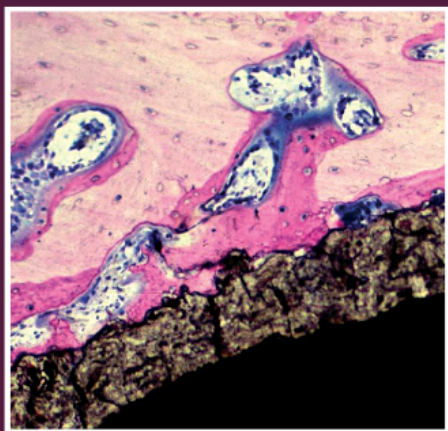


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Biocompatibility and performance of medical devices

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Published by Woodhead Publishing Limited,
80 High Street, Sawston, Cambridge CB22 3HJ, UK
www.woodheadpublishing.com
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PA 19102-3406, USA

Woodhead Publishing India Private Limited, G-2, Vardaan House, 7/28 Ansari Road,
Daryaganj, New Delhi – 110002, India
www.woodheadpublishingindia.com

First published 2012, Woodhead Publishing Limited

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British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library.

Library of Congress Control Number: 2012947092

ISBN 978-0-85709-070-6 (print)

ISBN 978-0-85709-645-6 (online)

ISSN 2049-9485 Woodhead Publishing Series in Biomaterials (print)

ISSN 2049-9493 Woodhead Publishing Series in Biomaterials (online)

The publisher's policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp which is processed using acid-free and elemental chlorine-free practices. Furthermore, the publisher ensures that the text paper and cover board used have met acceptable environmental accreditation standards.

Typeset by Newgen Knowledge Works Pvt Ltd, India

Printed by TJ International Ltd, Padstow, Cornwall, UK

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Ever since manufactured medical devices were introduced to the public there has been a debate as to how those members of the public who actually receive a device can be assured that, on the one hand, their devices are fit for purpose and how, on the other hand, the devices are not going to do them any harm. We want a prosthetic heart valve to significantly improve the hemodynamic performance of the heart without giving any attendant increase risk of potentially fatal blood clots. We want a hernia repair mesh to effectively support the relevant connective tissue–muscle complex without producing any significant risk of tissue erosion or infection. Any device that utilizes an animal tissue in its construction in order to optimize performance should not simultaneously introduce unacceptable hypersensitivity risks, and so on.

Ensuring that the benefit–risk ratio for medical devices is extremely high, for that is what the patients want, is not a trivial task. Although those involved with the industry know that millions of patients receive entirely satisfactory performance with their devices year-in, year-out, over protracted periods of time, it is also recognized that, because patients are individuals, their responses to devices will not always be the same, so that some do not have full satisfaction. The same could be said for the variable quality of clinical skills, which again results in some poor performances and, yes, sometimes the devices are themselves, in spite of much testing, not always ideal. To talk of 90% success over ten years may give a very good perspective of the value of medical devices but, if you are one of the unlucky patients for whom a device fails, that is a 100% failure as far as you are concerned.

The solution to this conundrum lies in scientifically-based, clinically-predictive testing of the performance and safety of the devices and biomaterials. The problem with this solution is that, whatever scientific principles we use, it is very difficult to devise a test system that accurately replicates the conditions within the human body, so predictiveness is never going to be guaranteed. There are also diverse, and often competing, interests at play here. It is necessary, for example, to respect the views of ethicists who argue against the use of animals in product testing, so that we often end up with using animals but in minimal numbers, which usually means that statistical

analysis is rather meaningless. Then we have, quite understandably, the commercially sensitive position of manufacturers who often have to insist that the results of their tests are confidential so that equivalent materials are tested time and time again under confidential conditions, the results never finding their way into the public domain.

It is no wonder that this testing arena is fraught with difficulties and inconsistencies. This is the essential rationale for this book, in which multiple authors, with varied experiences on the sides of the manufacturing industry, the regulatory bodies and the test houses themselves, have come together to present their views on the best options, the best techniques and the optimal methods of interpretation of data. This collection has been expertly assembled by Jean-Pierre Boutrand, who has many years of experience in one of the best-known test companies.

None of us can pretend that there is uniformity of opinion within these chapters, and this is not unexpected. However the ground is extensively covered and the reader should get a thorough knowledge of the principles and practices, and of both the benefits and difficulties of interpreting these test procedures. The reader will even find that there are some differences of opinion on what biocompatibility and biological performance actually mean, and what type of test should be performed and what should not be done. There are some very difficult issues that arise when the test data produced lie just on one side or another of what is, we all have to agree, a rather arbitrary pass–fail criterion; are the best interests of the patient (and indeed other stake-holders such as insurers and doctors) being served by a strict adherence to failure criteria or by a little leniency, since, after all, a failure to allow a ‘good’ device onto the market because of a marginal failure in an inappropriate test, means the denial of ‘good’ devices to patients. Hopefully the contents of this book will allow the reader to attain an insight into the complexities of this testing arena, and inform society in general of the sincerity and professionalism of this arena.

David Williams

Medical progress resonates with innovation.

True innovators are learners, not knowers. Hence true innovation is associated with complex cycles of discovery, change, failure and, subsequently, sharing of knowledge.

Considering medical device development, while failure is part of the process of improvement and may appear inevitable or even worthy, it is not acceptable at the stage of 'first in man' for obvious ethical reasons.

The consequences of failure at the stage of clinical use of a medical device are so severe that, if the probability of failure cannot be properly characterized, this should either slow down the device-development cycle and retard market access or limit the availability of potentially beneficial innovation.

To counteract this situation, non-clinical safety and performance evaluations play a key role in ensuring that patients treated with a new medical device are not placed at undue risk, and in providing reasonable probability that the new device will offer the expected level of efficacy in humans.

To achieve this goal, multidisciplinary teams should join their efforts to design, produce and approve non-clinical safety and performance studies that are ethical, scientifically sound and compliant with applicable regulatory and quality standards. As illustrated by the variety of origins of contributors to this book, these teams include individuals with mechanical, chemical, material, biological and medical backgrounds, and experience from the medical device industry, contract research or academic laboratories, notified bodies and governmental agencies.

To help reach this goal, this book aims to offer both strategic and practical advice to assist the reader in developing safe and effective medical devices while shortening their development cycles.

Jean-Pierre Boutrand

To my wife Severine, who encouraged this project and was sure the team
of assembled writers could do it together.

To those who understand that progress of any sort advances through
research, innovation and calculated risk-taking.

To those who strive to make the best use of science in order to improve
and protect human lives.

To medical device inventors, developers, manufacturers, quality
professionals, regulators and users: keep on supporting innovation ...
there are so many needs for new devices!

‘If you think you can or think you cannot, you are correct’ – Henry Ford.

Concepts in biocompatibility: new biomaterials, new paradigms and new testing regimes

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Abstract: In this chapter the traditional methods to assess biological safety are placed in the context of a new way forward with test procedures that reflect the biomaterials of the future, since both our understanding of biocompatibility phenomena, and the uses and expectations of these new biomaterials, are undergoing radical change. The main issue here is that, historically, biomaterials have been designed to be as inert as possible, but this approach is of limited value in applications that demand some form of biological functionality. The problems of existing *in vitro* and *in vivo* test procedures are explained within the framework of a new understanding of the mechanisms of biocompatibility.

Key words: biocompatibility, biological functionality, implantable medical devices, tissue engineering templates, nanoscale diagnostic systems.

1.1 Introduction: traditional biomaterials and biocompatibility test procedures

Biomaterials science is now over 50 years old. Although surgeons had already been using various synthetic and natural materials for a variety of procedures for a few centuries, mostly to facilitate tissue repair, the real impetus for the development of materials that were intended for use in the body came in the late 1950s and 1960s with the realization that parts of the body could be effectively replaced by synthetic objects. Quite soon a significant industrial sector had been formed and millions of patients were having their quality of life improved through the performance of these materials.

The emergence of this industrial sector brought significant challenges as well as opportunities. These included the need to ensure, as far as possible, that the materials and the devices made from them were safe and effective. In the very early days of the development of implantable medical devices, it was quite possible for surgeons or physicians to construct their own devices, often in their own private workshop, from a variety of commercially available

commodity materials, where the liability considerations were minimal and covered by their own medical license. When devices were manufactured by a commercial enterprise, this no longer applied and attention was focused on the need for some oversight of this new industry. Regulatory agencies were established in some jurisdictions, and legislation emerged concerning products that were allowed to be sold and used clinically.

Questions naturally arose here as to what governs safety and efficacy, and how such qualities could be determined prior to the devices being used in human patients. Various tests were developed and promoted in order to determine functional performance of some devices, and performance standards and evaluation procedures emerged and were used in the pre-clinical determination of putative effectiveness. Whether or not a material or device could be considered safe was a more challenging issue, especially in the context of the possible responses of the human body to such devices. The poor scientific understanding of the mechanisms by which tissues could respond to implanted materials exacerbated these difficulties. This subject was eventually given the name 'biocompatibility' and a variety of test procedures were introduced in order for biomaterials to be assessed with respect to their biocompatibility. In the context of product testing, as opposed to scientific experiment, the subject became more widely, and correctly, described as biological safety, an aspect to which we shall return later.

Over several decades, major activities and programs on a worldwide basis have resulted in arrays of test procedures to determine biological safety with respect to biomaterials and their associated health-care products; these tests constitute the subject matter of this book. What I wish to do in this chapter is to place these endeavors into the context of the way forward with test procedures of the future, since both our understanding of biocompatibility phenomena, and the uses and expectations of these new biomaterials, are undergoing radical change.¹

At the heart of this debate is a fundamental change in what we expect biomaterials to do. We may take some examples of conventional implantable devices in order to identify what we expect of them. With total joint replacement prostheses, we require functionality through the effective transmission of forces between the articulating components. We expect this functionality to last for many years, during which time the prosthesis remains attached to the relevant areas of bone and does not produce any adverse response from adjacent or remote tissues that could compromise either the performance of the device or the health of the patient. The functionality is not primarily a biological safety issue. However, there are a few very important factors that control the response from the patient, and these relate to the overall biocompatibility of the material components. These could depend, for example, on corrosion, degradation or leaching phenomena, or the specific biological response to the wear debris that is generated at the articulating

surfaces. As we introduce different materials that are intended to facilitate fixation of prostheses to bone, as with bone cements or so-called bioactive coatings, we bring new requirements with respect to their own biological performance, but ultimately they have to perform their own fixation functions without adversely affecting the overall biological performance of the systems. We should bear in mind that cements function mechanically and not biologically, and that the biological performance of bioactive surfaces is limited to a marginal effect on the rate of new bone apposition, which may itself be counterbalanced by eventual degradation, resorption or delamination. In other words, the biological safety of joint replacement prostheses is determined by the reactivity of the materials and any derivatives, such as wear particles, metal ions and monomers, and that the best performances are likely to be achieved with materials that are as chemically and biologically inert as possible.

With intraocular lenses, the functional requirement is light transmission and the biological requirements are based on the need for the lens material to irritate eye tissues the least so that there is no reduction in light transmission through interference from a localized inflammatory response. Again the most desirable materials have good optical clarity and maximum chemical and biological inertness. With pacemakers, implantable defibrillators, deep brain stimulators and other such devices, the material for the casing simply requires to be acceptable to the local tissue without significant response, the lead insulation requires good insulation functionality coupled with resistance to degradation and minimal tissue responses, and the electrode tip needs to minimize the fibrosis that could affect the ability of charge transfer across the interface. Again we are seeking maximally inert surfaces. With prosthetic heart valves, the functionality is solely concerned with the control of blood flow, which has to be achieved without stimulating the clotting of blood or impairing the healing of surrounding tissue, these features being achieved by chemically and biologically inert materials.

The conclusion from these and many other examples is that the traditional biomaterials used in the more conventional implantable medical devices have to be inert, displaying minimal reactivity with the components of the tissues of patients. It is of no surprise that the inventory of acceptable biomaterials has reduced in size over the years and we now have a group of the most corrosion-resistant alloys (titanium, cobalt alloys, platinum group metals), the most inert of oxide ceramics (alumina, zirconia), the most biostable polymers (polyethylene, polypropylene, polytetrafluoroethylene (PTFE), polyether ether ketone (PEEK), silicone elastomers, some acrylics) and some highly inert carbons. In those situations where biodegradability or bioactivity is sought, most solutions involve materials that are based on resorbable and metabolizable components, such as calcium phosphates and simple polyesters. In some cases, very specific functionality

requirements suggest that these materials may not be the most appropriate, such as those requiring shape memory behavior, but even here choices are made on the basis of the most inert of those materials that have such functionality.

These characteristics have dominated the ethos of pre-clinical testing of biomaterials with respect to biological safety, where the principal requirement has been that the biomaterial should interact minimally with the tissues of the human body. There is very little that is positive about biological safety tests; virtually all of them are aimed at a series of negatives determined by this lack of interactivity. In other words, biomaterials are determined to have passed biological safety tests if they are shown, in a range of *in vitro* and *in vivo* tests, not to be cytotoxic, genotoxic or carcinogenic, and not to initiate any form of ‘adverse’ local or systemic effect, any irritation or hypersensitivity, or any activation of blood components.

There are three problems with this approach. The first is the clinical relevance of such tests and the significance of any pass/fail, or go/no-go limits are not scientifically based; I shall return to this later. More importantly, the underlying assumption that the biomaterials should not interact with their biological environment in order for them to be ‘biologically acceptable’ or ‘suitable for clinical use’ may be entirely inappropriate. The basis of this assumption lies with the changes in biomaterials applications, and indeed the fundamental concepts of biomaterials science, which are covered in the next couple of sections. Thirdly, many of the test procedures rely on the generation of extractable, or leachable, components and the evaluation of the responses to such components *in vitro* and *in vivo*. This is not necessarily appropriate with many of the newly emerging biomaterials and health-care products.

1.2 The evolution from implantable medical devices to regenerative medicine and bionanotechnology

While implantable medical device technology has had many successes, there are several generic limitations. The main limitation has been concerned with the fact that, as implied above, these devices have been intended to replace diseased or injured tissues by mechanical or physical functionality. This works well for several situations within the musculoskeletal and cardiovascular systems, and in some sensory organs, but does not, and cannot, address the majority of diseases and injuries. Traditional medical devices do not function primarily through biological or immunological mechanisms. They are not intended to facilitate regeneration of the affected tissue, nor are they expected to interrogate those tissues through any sensing or imaging modality. All of this has been changing in recent years, and we are witnessing

an evolution from implantable devices that replace tissues to those systems that are directed at tissue regeneration, cell manipulation or the assessment of tissue ultrastructure and function.² It should be intuitively obvious that the biomaterials used in these new technologies would not be expected to function by being as chemically and biologically inert as possible.

The significance of this observation can be seen with a few powerful examples. Tissue engineering is one of the technologies of regenerative medicine. Although the definition of tissue engineering has been evolving in recent years, my own definition covers the essential scientific features; tissue engineering is 'the creation of new tissue for the therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells through a systematic combination of molecular and mechanical signals.' This definition contains the words 'deliberate,' 'controlled' and 'systematic' in the context of regenerating new tissue, all of which imply that highly specific biological processes are required. Moreover, it is intended that these processes involve the specific delivery of signals to cells. For example, the target cells may be bone marrow derived mesenchymal stem cells (MSCs) and we may wish to use these as the basis for the creation of, say, new cartilage tissue. We require this new cartilage to have a specified size and shape, and for this purpose we create a biomaterial template, sometimes called a scaffold. Into this template we introduce the MSCs. By themselves these are not going to generate cartilage. Instead we have to help them to differentiate into chondrocytes, which are the cartilage-producing cells, and for this purpose we add a chondrogenic growth factor, and possibly we alter the genetic structure of the cells through delivery of a specific gene to the MSCs. Again this is unlikely to be sufficient and so the cell and growth factor laden template is placed in a bioreactor, where a cell culture fluid medium, containing appropriate nutrients, is forced to circulate in order to provide fluid shear stresses to the cells. Structural compressive stresses may also be applied. It is through this combination of structures and chemicals that the appropriate signals are applied to the MSCs, which, if the conditions are right, can generate new cartilage.

Within this new form of medical technology we have a biomaterial-based template. Questions naturally arise as to what this biomaterial should be, and how we should determine its suitability for the functional but safe generation of new tissue. In other words, what should be the specifications for this biomaterial? Added to the potential list of requirements of the biomaterial, we have to take into account the usual expectation that the material will be biodegradable such that the tissue being regenerated takes over, or replaces, the template over time.

Given these requirements, we should not expect that any of the classical medical device biomaterials discussed in the previous section would be appropriate. There are two reasons for this. First, they have been developed

in order to have no interactions with the tissues of the body, which should certainly preclude them for consideration with respect to the templates that deliberately and systematically carry out biological functions when they stimulate cells in a controlled manner. Secondly, the vast majority of them have been designed to be as inert as possible rather than be degradable. The vast majority of biomaterials that have been used as scaffolds/templates have been selected by processes that totally ignore the first of these factors but which embrace those materials that have had prior regulatory approval in biodegradable medical devices, such as surgical sutures and some simple drug delivery systems. The vast majority of biomaterials used in the early stage commercialized products in tissue engineering were based on the principal specification that the material had to have prior FDA approval for use in traditional medical devices. Whilst being a pragmatic approach, this was hardly scientifically based and has led us in entirely the wrong direction for the design of tissue engineering template biomaterials.

A similar situation may be seen with biomaterials-based systems in delivering molecular or nanoparticulate agents to cells, either for therapeutic or diagnostic purposes. Examples here include superparamagnetic iron oxide nanoparticle magnetic resonance imaging (MRI) contrast agents, cationic polymers as non-viral gene vectors, carbon nanotubes filled with chemotherapeutic agents and functionalized carriers for immunotherapeutic agents. None of these systems will work if the material carrier or agent is ignored by the target cells; instead they have to be attracted to them or possibly bind to highly specific receptors on cell surfaces. In many situations, the agent has to pass through a cell membrane, negotiate its passage through the cell cytoplasm and deliver an active agent to the nucleus or to organelles.

In these applications we are often dealing with potentially toxic agents, so their use is dependent on precise dosage and precise targeting. It is of no value whatsoever to determine if an *in vitro* derived extract from the biomaterial is cytotoxic within a culture of fibroblasts. We need, therefore, a different perspective on the topic of biocompatibility if we are to understand the mechanisms by which the new range of biomaterials interact with the hosts in which they are placed,³ and if we are to assess the nature and significance of these interactions as far as the safety of the patient is concerned.

1.3 New concepts and definitions for biocompatibility

Ever since biomaterials were first used within the human body, it has been accepted that interactions take place between the materials and the components of the body, and that these have a significant impact on the ability of that material to satisfactorily perform the desired function for the desired length of time in that body. It has become obvious that the nature of these

interactions varies from one clinical situation to another. In reality, there is not just one overarching type of interaction that could take place; there are very many of them, with a myriad of potential individual and interconnected reactions. For many years these different phenomena have been discussed collectively under the broad heading of biocompatibility, although few people have had any idea of what biocompatibility really meant and we are still largely ignorant of the precise details of these mechanisms.

As we have seen, biocompatibility was first discussed in the context of implantable medical devices and early definitions of the term reflected this situation. Probably the most widely accepted definition of biocompatibility was derived in the 1980s, and states that biocompatibility '*refers to the ability of a material to perform with an appropriate host response in a specific application*'. This, of course, is a conceptual definition rather than one of immediate practical usefulness, but indicates three important factors; (a) the biomaterial has to perform and not simply exist, (b) out of all the possible reactions and responses from the tissues of the body, the response that is the most appropriate for the application in question is required, and (c) we always have to define biocompatibility in the context of the specific application.

The latter point is extremely important since it implies that, for a given material, the biocompatibility characteristics vary from situation to situation. Biocompatibility is not, therefore, a property of a biomaterial; it is a characteristic of a material–tissue system. The corollary of this is that there is no such thing as a biocompatible material, a fact that is still lost on the majority of biomaterials scientists and which is at the heart of a number of biomaterial failures.

We have noted above that many of our ideas about biocompatibility have had to change in recent years as new and varied biomaterials applications have been introduced. New definitions have had to be introduced, and a discussion about the mechanisms of biocompatibility today has to be quite different to those of just a decade ago. There are two very important fundamental facts that underpin biocompatibility phenomena. First, with very few exceptions, we find that when man-made, engineering or commodity materials are used as biomaterials (which accounts for the vast majority of biomaterials used to date) they are not intrinsically compatible with physiological systems, nor have they been designed to be so. Secondly, the tissues of the human body have not evolved in order to benignly accommodate these materials within their midst and they will be treated as 'foreign' and 'potentially harmful.' The default position, therefore, is that there is inherent incompatibility between these two compartments, the biomaterial and the tissue. But it is much more serious than that; the human body has evolved in such a way as to have exquisite detection mechanisms that readily identify foreign objects (historically in the form of microorganisms), and

equally important, there are exquisite defensive mechanisms that deal with such objects once they have been detected; we are confronted, therefore, with an active incompatibility and not a passive one. These mechanisms evolved naturally to deal with bacteria and viruses, but they are often capable of diversion towards any synthetic material that might find its way into the body or any type of biological stress that may arise with this use. This becomes especially important when we design biomaterial components that have some similarities with the bacteria and viruses, both in size and chemistry, so that we are inviting these reactions to take place and have to devise means to avoid them.

Many synthetic materials are susceptible to degradation within aqueous environments. The tissues of the body are aqueous-based, and have a collection of species, both cellular and molecular, that are mobile and aggressive, so that the already corrosive environment is powerfully enriched by these active agents. Thus, aggressive host–biomaterial interaction mechanisms are readily available as soon as the physician exposes the former to the latter.

When a material is placed within tissues, the response will evolve over time, depending on the physical characteristics and biostability of the material, and it may change with the circumstances within the body. The evolving response has often been referred to as the foreign body response, especially with implantable devices, although this is a rather imprecise term. Clearly the nature of this response will depend on the specific characteristics of the material behavior and of the host tissue behavior. There are a few general principles that we have to bear in mind.

- *Chronology*: The mechanisms of biocompatibility do not show linear progression with time. In many situations, usually on the side of the host, one event may be triggered spontaneously, at any time, the effect of which can be powerfully amplified by one or more mechanisms, changing the whole nature of the response in a short space of time.
- *Location*: The consequences of the interactions between material and host may be localized to the vicinity of the material, giving the local foreign body response, or they may be remote, affecting either the whole body (a systemic response), or a specific discrete remote site, for example the site where a corrosion or degradation product is eventually stored. Local foreign body responses are very important in the area of implanted medical devices. Systemic effects can occur with any biomaterial but have taken on greater significance as molecular or nano-scale biomaterials have been introduced for drug and gene delivery or for imaging contrast agents, where the products themselves are highly mobile and often delivered by injection rather than surgically.
- *Controlling factors*: Although biocompatibility is obviously controlled by the nature of the material, device or agent, it is clearly influenced by many

other factors. Biocompatibility phenomena vary from patient to patient, and with the techniques used to administer the biomaterial to the patient.

Biocompatibility is therefore a series of individual phenomena that combine to generate the overall host response. Since there are so many variables that may affect each of these components and the mechanisms that are associated with them, it should not be surprising that there are an almost infinite number of possible features of the host response. The original definition gave a powerful reminder that biomaterials have to perform a function, and can only do so if they invoke a response from the tissues, or tissue components, that they are in contact with, that is, at the very least, compatible with that function, or better, actively support that function. It is necessary, as originally envisaged, to define biocompatibility specifically in relation to those functions, but also to have a more profound overarching concept.

It is clear from some well-established situations, in which there is ample clinical evidence, that the principal component of a material's biocompatibility is that, whatever the desired function, the material shall do no harm. Long-term implantable medical devices are obvious here, and the following definition or paradigm may be proposed:

The biocompatibility of a long-term implantable medical device refers to the ability of the device to perform its intended function, with the desired degree of incorporation in the host, without eliciting any undesirable local or systemic effects in that host.

We will note later that this definition does not state, nor indeed imply, that undesirable effects have to be chemically induced. This is an important issue since, as noted above, many biological safety test procedures are based on the effects that extracts or leachables derived from biomaterials have on biological components, especially cells. This whole approach is predicated on the assumption that biological safety is exclusively determined by the chemical nature of easily extractable components; this is patently not the case with many biomaterials.

The above definition suggests that we may wish to consider biocompatibility with an overarching concept but with different themes that vary with the circumstances. The edict that the biomaterial shall do no harm may well be reassuring to the recipient of an implantable device that is intended to survive longer than them, but may not be sufficient for other stakeholders in advanced medical technologies, where specific functionality, usually sooner rather than later, as well as safety is required. As noted earlier, if we take tissue engineering scaffolds as examples, there is little point in using inert materials, or, even more importantly, materials that have non-specific or inappropriately directed activity. Here, it is suggested that a more appropriate paradigm would be:

The biocompatibility of a scaffold or matrix for a tissue engineering product refers to the ability to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signaling systems, in order to optimize tissue regeneration, without eliciting any undesirable local or systemic responses in the eventual host.

The unified concept of biocompatibility is therefore as follows. A biomaterial is, by definition, foreign to the host, whether that is the recipient of a surgically invasive device, of a construct for the purposes of regenerative medicine, a drug or gene delivery entity or a vehicle to assist in diagnosis or imaging. Whatever the required function or purpose, the device or construct, and therefore the material of its construction, shall not produce any clinically significant adverse effects in the patient or host. However, the material should be expected to produce demonstrable beneficial effects in that host, whether that be the stimulation of specific differentiation in stem cells, the positive assistance in the maintenance of cell phenotype, the facilitation of endothelialisation of intravascular devices or the pharmacological control of undesirable responses, such as intimal hyperplasia associated with intravascular stents and osteolysis associated with the release of wear debris from joint replacement prostheses.

We may therefore redefine biocompatibility as follows:

Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy.

Our understanding of the mechanisms of biocompatibility has been restricted whilst the focus of attention has been long-term implantable devices. Here, over 50 years of experience has determined that, in the vast majority of circumstances, the sole requirement for biocompatibility in a medical device intended for sustained long-term contact with the tissues of the human body is that the material shall do no harm to those tissues, achieved through chemical and biological inertness. Rarely has an attempt to introduce biological activity into a biomaterial been clinically successful in these applications. Only now that the focus for biomaterials has turned towards tissue engineering, sophisticated cell, drug and gene delivery systems and indeed applications in biotechnology, has the need for specific and direct interactions between biomaterials and tissue components become necessary, and with this a new paradigm for biocompatibility has emerged. It is believed that once the need for this change is recognized, so our understanding of the mechanisms of biocompatibility will markedly improve, as will the ability to evaluate these mechanisms.

1.4 A proposed conceptual framework for new biocompatibility concepts and testing regimes

The above sections have discussed the evolving concepts and definitions within biocompatibility and some of the ways in which the changing scenes may have an impact on how we assess biocompatibility and biological safety. We now come to the crucial point of identifying the more troublesome concepts and suggestions for ways in which a better understanding can be used in order to develop more effective methods of assessment.

1.4.1 Unifying biocompatibility mechanisms but different clinical outcomes

At the heart of our difficulty in understanding biocompatibility, and deciding how to assess it, is the misconception set out above that biocompatibility is a property of a material. It is not, and as emphasized so strongly, the idea that we can describe a material as being biocompatible is fundamentally wrong. Whilst everyone in the industry knows that biocompatibility/biological safety should be assessed in the context of the intended application, in reality a series of *in vitro* and *in vivo* tests are performed on a material according to a reputable standard, and if it should satisfy the requirements of the tests then it is deemed to possess biological safety. Once such an accolade is derived, then that material is usually assumed to offer safety in wide-ranging applications, and is often labeled, erroneously, as an FDA-approved biomaterial, or a CE-marked biomaterial, with implications of generic applicability rather than device- or application-specific utility.

From a practical perspective we should never assume that any one clinical application is the same as another and no simple set of *in vitro* and *in vivo* tests can demonstrate safety and utility across a range of such applications. No one *in vitro* cytotoxicity test can be equally relevant to the materials used for an indwelling catheter, a mechanical heart valve, a cochlear implant and a xenogeneic scaffold used for skin tissue engineering. No one material can be considered 'equally biocompatible' in any of these, or indeed similar, applications. It also appears illogical to use the same criteria for satisfying some criteria (which are often arbitrary from a biological perspective) for different types of health-care product.

It follows that biocompatibility testing should be specific to the intended application and not either to the biomaterial class or to any defined biological event. I suggest, therefore, a radical change to the processes where we take the results of simple *in vitro* and *in vivo* tests and use these to determine the suitability of materials for different and wide-ranging clinical applications, where we expect, and indeed require, different clinical outcomes.

Paradoxically, the fact that we should have different clinical outcomes with different biomaterials used in different circumstances does not mean that we have entirely different and unconnected mechanisms that control the interactions between the biomaterials and tissues in each situation. On the contrary, we should expect there to be a common set of mechanisms upon which all biocompatibility phenomena are based. As I have argued and explained in detail elsewhere,² it is possible to identify these underlying mechanisms that control all events, ranging from nanoparticle toxicity to thrombogenicity, osteolysis and stent-related intimal hyperplasia. I group these events within a pattern of biocompatibility pathways. Each pathway has one or more biological markers, which should ultimately allow us far better methods of determining, and even predicting, these biocompatibility phenomena.

1.4.2 Embracing the new concepts of biocompatibility

The classical concept of the biocompatibility of biomaterials, especially for those used in implantable devices, has involved the development of a host response that consists of phases of inflammation and fibrosis and which, in the putative ideal case, results in a quiescent cellular zone around the material with a fibrous capsule of minimal thickness and activity. We assume that the mediators of any deviation from this pathway are the leachable components, so that our evaluation of biocompatibility, and biological safety, has to involve the detection of such components and the analysis of the ways in which they can perturb biological processes.

This is no longer acceptable, for several reasons. First, the majority of biomaterials in use today are not applied to the body by surgical implantation so that concepts of biocompatibility that are predicated on modifications to a wound healing process are no longer relevant. Second, we can no longer assume that the biological events in biocompatibility phenomena are controlled by the chemistry of leachable or extractable components. It is true that all biological events involve chemical signaling processes but this does not mean that they are chemically driven. Two powerful, non-chemically driven processes should be mentioned here, the first being mechanotransduction effects that are controlled by mechanical forces and the second being biophysical events that are controlled by the thermodynamics of macromolecular adsorption on biomaterials surfaces. Third, we have to recognize that biological events related to biomaterials within physiological environments are scale dependent, and mechanisms may not always be equally applicable at the macro-, micro- and nano-scales. We have to embrace the new concepts of biocompatibility and use these as the basis for our procedures of evaluation.

1.4.3 Problems with *in vitro* tests

I have already alluded to the difficulty with standard *in vitro* tests that rely on extraction procedures. It is true that with many traditional implantable devices and the materials from which they are constructed, low molecular weight species, for example metal ions, monomers and ionized products of ceramic degradation may be released from a material surface, with the potential to participate in biological reactions. Equally, many commercial biomaterials contain additives or impurities that may also be released, with similar consequences. Under such circumstances, it is sensible to determine, within appropriate experimental systems, the extent of this potential. This could involve cytotoxicity, genotoxicity and other tests. However such tests have significant limitations. Many readers will be aware, from either industry or regulatory sides, that the results of such tests can be rather indeterminate, or equivocal; materials that fail to satisfy the requirements may be tested again and meet the same criteria, or different endpoints may be used. It may be strongly argued that many failures of implantable medical devices derived from biocompatibility deficiencies have occurred in device/material combinations that had received regulatory approval after successfully meeting the requirements of standard *in vitro* pre-clinical tests. Moreover, extraction or leachable tests may have some applicability with monolithic objects but are less appropriate, or indeed highly inappropriate, for other materials, especially those at the nanoscale.

1.4.4 Problems with *in vivo* tests

There have been extensive discussions about the relative merits of *in vitro* and *in vivo* procedures for biocompatibility evaluation. There is no simple answer to such questions although there is a general consensus that *in vitro* tests give more accurate data about individual biological processes but that *in vivo* tests are more predictive of clinical use. Within the latter domain, the really important questions arise over the choice of animal model with respect to species, strain, gender, age, anatomical site and other factors. The vast majority of tests are performed on rodents, with decreasing numbers on rabbits, dogs, calves, pigs, sheep and primates. Difficulties abound with all species, ranging from the lack of human relevance with small mammals and the costs and ethical/legal dimensions of working with non-human primates. Tests may be performed on rats that show either or both carcinogenicity or reproductive toxicity through mechanisms that are inoperative in humans. On the other hand, it is possible to see quite different outcomes with similar procedures and similar materials in pigs, goats and sheep. Some biological outcomes with large animals have not been predictive of performance in

humans. As we move to procedures in regenerative medicine, it is becoming clear that the function of cells varies considerably with gender and age. One thing is very clear; many *in vivo* test procedures are simply non-predictive of performance in humans and there is little scientific or clinical sense in pretending that they are.

1.5 Conclusions and future trends

This is an essay that sets out a plethora of problems that face us in our understanding of biocompatibility concepts and the methods by which we assess the biological safety characteristics of biomaterials, preferably in advance of their clinical use. I show here the directions in which we should move, but clearly do not have all of the answers at this stage. It is necessary to emphasize strongly, however, that, as I write this towards the end of 2011, we are faced with a public outcry about the apparent lack of safety associated with many medical devices. This includes metal-on-metal hip replacements, meshes for the treatment of pelvic organ prolapse, breast implants and many others. We have to admit that we, collectively, do not have the correct procedures even for those types of device that we have been using for many years, let alone the new types of device and procedure we are just embarking on. It is obvious that tissue engineering has not yet resulted in routine clinically successful procedures and this is partly due to our failure to understand the critical biocompatibility characteristics of scaffolds and templates. We should be reminded of the massive safety issues that arose a decade ago with clinical trials of gene therapy using viral vectors as we rush into experiments with non-viral vectors. And we should be acutely aware that the immense promise of the medical uses of materials at the nanoscale has to be weighed against the uncertainties over biological events that can occur with such nanomaterials.

I suggest, therefore, a radical rethink of the way in which we assess the biological safety of biomaterials and the way in which we use the outcomes of test procedures within the regulatory process. Tests have to be far more oriented towards specific clinical applications. We cannot carry out classical cytotoxicity tests on tissue engineering scaffolds and templates and assume that the results will be informative about tissue regeneration processes. We cannot carry out simple *in vitro* studies on quantum dots and use the results to determine their safety in diagnostic procedures.

In addition, there should be a much closer relationship between academic studies of biocompatibility and the regulatory testing of biological safety. The results of the former simply do not inform the procedures of the latter at this stage. Most simple *in vitro* tests should be abandoned unless they can demonstrate reliable and reproducible clinical relevance. We should re-examine the *in vivo* tests that are performed. Most current procedures

are qualitative and statistically unfounded. Many, as described earlier, are incapable of predicting performance in humans; many simply do not have scientifically valid endpoints.

Two immensely important points should be emphasized here. The first is that we have to embrace new technologies if we are to make serious progress, even if we recognize that these are long-term options. Biomaterials science is already using high-throughput techniques for polymer design and the interrogation of cell–material interactions. There have also been attempts to use genomic techniques, especially gene expression profiling, in the response of cells to materials. Their use has to be enhanced and accelerated. The future goal has to be the complete understanding of biocompatibility pathways and the use of computational models to predict how new biomaterials will perform within these pathways.

The second is that the biomaterials industry should be far more open with patient populations about risks and risk–benefit analyses. Patients should be made aware that there are no guarantees of safety and effectiveness, and they should be informed of patient, and indeed clinical, factors that can control success or failure as much as the device itself. The consequence of this is that far more attention should be paid to follow-up analysis of performance and safety. There are systems for post-market surveillance and there are some implant registries, but these are not sufficiently informative at the present time to make much difference in a prospective fashion. The arguments about costs and patient confidentiality have been around for a long time and have to be addressed far more robustly if we are to generate a safer market place.

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Challenges in biocompatibility and failure of biomaterials

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Abstract: The extensive use of medical devices for the treatment and prevention of acquired, inherited, traumatic or degenerative lesions in the body generated a tremendous need for technologies and materials under the control of regulations, using standards as tools. Biocompatibility, largely described in other chapters, is one of the prerequisites for the use of medical devices. However, the harmful effects of medical devices after several decades of use should also be considered to complete our knowledge and understanding of biocompatibility.

Key words: biocompatibility, standards, vigilance reports, medical devices.

2.1 Introduction

Medical devices (MDs) and biomaterials have been employed empirically for more than 2000 years but their use has dramatically increased during the last century, essentially over the last 40 years. In response to an ever increasing demand for improved quality of life in a steadily aging population, improved technologies in metal, ceramic and polymer science have opened up new areas for MD applications in orthopedy, cardiovascular assistance/repair, general surgery, and wound healing. It has been acknowledged that in this rapidly evolving domain, the mean ‘industrial life’ of a MD is about five years.

Engineers, chemists and medical doctors are jointly developing new concepts and products and their challenge and that for the Regulatory Authorities is to ensure the safety and the appropriate performance of MDs. Despite considerable progress in the development of test standards for the evaluation of biological responses and notwithstanding a general consensus about the efficacy of existing procedures, the predictive value of these tests and their objectivity is still challenged. It is the aim of this chapter to report and discuss critical examples of the complex biocompatibility concept.

2.2 Concept of biocompatibility

The concept of biocompatibility is analysed and completed by examples of harmful effects related to medical devices that illustrate by contrast the concept of 'bio-incompatibility'.

2.2.1 Appraisal of biocompatibility: safety and performance

Biocompatibility is a complex concept associated with the extensive domain of MDs use and to the multiple interactions related to their safety and performance.

The first component of the concept of biocompatibility relates to the definition(s), itself that have evolved through time, reaching a consensus in 1986 that is likely to be updated, or at least revisited, with regard to current scientific knowledge and technological developments.

In the Chester Consensus Conference (1986) on Definitions in Biomaterials, agreement was achieved on the following definition: 'Biocompatibility: the ability of a material to perform with an appropriate host response in a specific application'.

At that time, it was important for the participants at the Consensus Conference to highlight several essential issues related to biocompatibility, including:

- The concept of performance is clearly a component of biocompatibility.
- The existence of a host response is acknowledged; the response is an integral part of the process induced by the material placed in the living tissues. In the great majority of circumstances, biomaterials are not intended to interact with biological systems but rather to function mechanically or physically without doing any harm.
- This host response should be acceptable, although there is no threshold for acceptability. The host response should be examined both in terms of its local and systemic reactions, and with regard to safety and its intrinsic performance. The host response is also a dynamic process, where both immediate and long-term effects on safety and performance should be considered.
- Performance relates to a specific application, in a specific tissue, with specific design interfaces. Therefore, the widely used wording 'biocompatible material' should not be used without referring to the specific application for which evidence of biocompatibility has been obtained. Several examples exist to document that a given material, although biocompatible for some indications is associated with a reaction that makes it non-biocompatible for other indications; suture materials are an example.

2.2.2 Factors affecting biocompatibility

The second component of the biocompatibility concept is related to the great number of existing materials in use; more than 5000 varieties of MDs are already on the market or under development. Thus important factors include:

- The large variety of materials used as components, ancillaries or processing aids for MDs.
- The complexity of the composition for any single device. The term 'medical device' is wide ranging, at one extreme consisting of a single material which may however exist in more than one physical form (bulk, powder, coating, etc.), and at the other extreme consisting of a complex instrument or piece of apparatus, made of numerous components with 2–10 or up to 15 materials.
- The large variety of production processes for devices, often subcontracted to third parties, with sometimes relatively incomplete control over these processes and their changes. Consequently, unexpected and uncontrolled process residues may be present and contaminate the surface of the device.
- The large panel of different structures for the same material according to its final use and claims (woven, knitted, highly polished, micro structured, film, tube or sponge, etc.) inducing variable local tissue responses according to physical parameters such as smoothness, porosity, structure, rheology, etc.
- The broad spectrum of surface treatments/coatings available in order to address inertness or conversely bioactivity of the interface.
- The importance of interfacial phenomena as key factors of biocompatibility, for example, the cellular macrophagic activation on material holes/defects of 50 μm , the platelet and hemostatic activation in contact with micro-defects in the range of 1–10 μm on the surface of blood-contacting devices, the role of such micro-defects in the adhesion, the development of microorganisms and biofilm formation, the encapsulation of smooth breast implants versus texturized in addition to the well described specificities of chemical surface properties like hydrophilicity/hydrophobicity, surface energy, etc.

2.2.3 Evaluation and assessment of biocompatibility

The third component of the concept relates to the standards used to assess biocompatibility, taking into account:

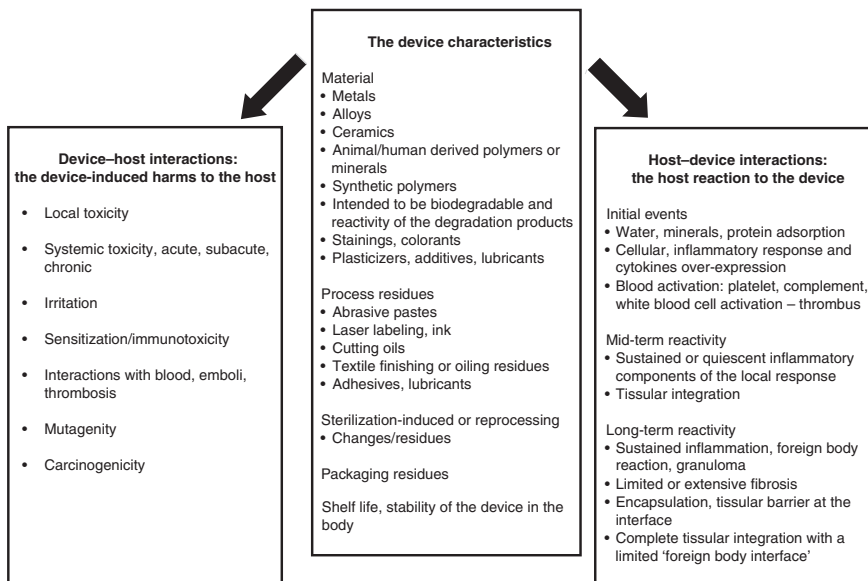
- Safety: this is generally addressed by the cohort of biocompatibility programs derived from the Tripartite Guidance Document and subsequent

International Standards (ISO 10993, EN 30993 series), which also include chemical characterization of the device, of its surface and extractables, recommendations for the analysis of degradation products and the establishment of dose-exposure limits based on a Risk Assessment Process.

- **Performance:** this is more or less covered by vertical standards that were developed for only a limited range of MD (intra ocular lenses, vascular prostheses, cardiac valves and stents, etc.) or by Regulatory Bodies' Guidance Documents (FDA).

These three components are summarized in Fig. 2.1, where the main characteristics of MDs are summarized in the center, the safety issues are gathered on the left and tissue responses and their consequences on the performances of the device/material are summarized in the right column.

Taking into account the strong interaction between MD characteristics, biocompatibility and claimed performances, ISO 10993-1 standard recommended that each device should be released after a process of risk assessment, under the clinical conditions of use, with a final statement about the risk/benefit for the patient. A risk management standard has been produced (ISO 14971) that addresses all dangers and risks associated with the use of the device. More recently, the last version of the ISO 10993-1 standard (2009) introduced the concept of a biocompatibility risk assessment based on all the different components, material characterization and potential



2.1 The biocompatibility concept.

risks, under the clinical conditions of use for each MD. This standard will be largely presented and discussed in following chapters, but it should be noted that it introduced the concept of an expert evaluation of the following:

- The strategy and program content of the biological evaluation of the medical device.
- The criteria for determining the acceptability of the material for its intended purpose, in line with the risk management plan.
- The adequacy of the material characterization.
- The rationale for selection or waiving of tests.
- The interpretation of existing data and the results of additional tests.
- The need for any additional data to complete the biological evaluation.
- Overall biological safety conclusions for the medical device.

2.3 Examples of device recalls or alerts during the last decade in which biocompatibility issues were considered

Although biocompatibility evaluation provides frequent examples of bioincompatibility of materials, the real challenge to address is whether the current methods/strategies are able to prevent major biocompatibility issues. One of the better known examples of harmful effects arising from orthopedic implantation concerned the extensive granulomatous responses seen with the polytetrafluoroethylene (PTFE) used by Charnley in his first joint prosthesis. Almost certainly the bulk PTFE used passed the cytotoxicity and ISO implantation tests. No test in the ISO standards would have predicted the granulomatous response to this very inert polymer, a response largely mediated by the size of the particles generated in the wear process.

Therefore, even though we have extensive experience of over 30 years of MD testing, it is important to take advantage of the lessons gained from understanding failures and use this knowledge to improve MD risk assessment and management. Some examples of failures reported in National Vigilance Databases (MHRA, AFSSAPS, FDA, MAUDE, etc.) are analyzed below, including whether their occurrence was predictable.

2.3.1 Metal-on-metal implants combination

This case, reported as a Medical Device Alert (MDA) by the Medicines and Healthcare products Regulatory Agency (MHRA) in June 2007, concerned a femoral head plus metal acetabular cup that had been on the market for more than ten years. Up to April 2007, one UK study reported 43 revisions (from a cohort of 637 hips associated with extensive periprosthetic soft tissue necrosis).

Some also involved late dislocation or periprosthetic fracture. Revisions were necessary 1–7 years after implantation. When stems were explanted, extensive corrosion was observed on the surface of many of them within the area of the cement mantle. This level of corrosion of cemented polished cobalt chromium molybdenum stems had not been previously reported.

More recently, an increased rate of revision for hip replacement implants, again with metal-on-metal surfaces, was reported and led to a reinforcement of clinical follow-up, and for patients with clinical symptoms (abnormal pain, swelling around the hip, deteriorating hip function or radiological abnormality) to measurements of cobalt and chromium ion levels in whole blood to identify patients that might require closer surveillance (levels of either metal ion above 7 ppb). Such an event also raises the issue of the genotoxic effect of the released species, as the mutagenic effect of hexavalent chromium or cobalt is well established.

This long-term harm was probably unpredictable through the biological testing program performed on the individual components of the implant, but should have been addressed through the risk assessment procedure.

2.3.2 Residues of solutions used for reprocessing medical devices

In 2004, MHRA issued a MDA concerning Cidex (MDA ortho-phthalaldehyde high-level disinfectant solution: OPA), used for reprocessing urological instruments that may have resulted in hypersensitivity in some patients with history of bladder cancer undergoing repeated cystoscopy. This was reported to have occurred in 24 patients out of approximately 1 million urological procedures worldwide. In this case, ineffective removal of residues and trauma associated with cystoscopy, particularly in male patients, may augment allergic reactions, however the bladder cancer population was not considered as being at a higher risk than a for general patient population. Actions proposed to reduce this risk were to avoid the use of this product if a validated alternative was available and, if not, to ensure that the manufacturer's instructions for an adequate rinsing protocol were respected in order to minimize disinfectant solution residues. This example illustrates that complete control of a device includes the instructions for use, reprocessing conditions and, potentially, an additional biological evaluation in the worst case of reuse conditions. Liability of the manufacturer covers the instructions for use and any harm resulting from the recommended reuse conditions.

2.3.3 Toxicological effects of degradation products

Trilucent® (soya-bean-oil filled) breast implants were withdrawn from use in the UK in 1999 after the MHRA identified concerns about long-term safety,

particularly in relation to the breakdown of the filler material. Breakdown products of the soya bean filler material were known to be genotoxic, and the presence of the genotoxic compound was confirmed in the filler material taken from women with Trilucent® breast implants. In addition, an increased risk of implant rupture was observed compared with actuarial rupture rate reported in the literature, raising the problem of possible weakening the shell in contact with the soya-bean-oil filler. This resulted in breast implant removal in over 3700 women out of approximately 4500 women implanted the UK. Analysis of the fibrous capsule surrounding Trilucent® breast implants provided evidence that breakdown products had reacted with the DNA in the fibroblasts and inflammatory cells of the capsule. No such reaction was detected in white blood cells from the systemic circulation and no atypical changes were observed in capsules. This example suggests:

1. That interactions between components of the device should be an integral part of the biocompatibility program.
2. That the shelf-life concept has to be applied to all characteristics of a device (sterility, packaging, mechanical and physical properties, toxicological evaluation in the worst case scenario of the risk assessment).

2.3.4 Opacification of intraocular lenses

Of 868 implanted hydrophilic intraocular lenses, from a single UK manufacturer, 233 (27%) have been explanted due to problems of opacification. The phenomenon appeared to be related to the migration of silicone from the packaging onto the surface of the lens. This was observed 12–36 months after implantation. Similar findings (due to surface calcification) were also reported for another manufacturer and were also related to silicone migration from the packaging. MHRA received no reports of opacification of lenses associated with a new packaging material. This case illustrates the interaction between the device and its packaging that may impair the biocompatibility in terms of device performance due to unexpected local tissue response. It also illustrates the need for specific pre-clinical implantation models that would reproduce the clinical conditions of use, such as addressed in vertical standards issued by the Committee for European Normalization (CEN). Since lens opacification is difficult to assess through subcutaneous or intramuscular implantation studies, the relevance of an intraocular lens implantation in a preclinical model to assess biocompatibility is evident. In addition, this example illustrates the critical nature of testing final products that have been packaged and sterilized under the conditions that will be used for human therapy.

2.3.5 Corrosion between adjacent devices

The special buccal environment, with salivary electrolytes (Cl^- , H^+ , HCO_3^-), poorly aerated areas in the sulcus, or well aerated surfaces at the occlusal surfaces, plays a determining role in the nature of corrosion phenomena observed. The electrochemical nature of the corrosion process is significantly influenced by combined mechanical stress, in addition to galvanic couples caused by using metal alloys of different nature. In the buccal environment, the clinical manifestations include gingival discoloring that may be associated with taste impairment and burning gums, gingival inflammation, repeated appearance of aphtaes, surface degradation of the restorations, breaking of the prostheses and recurrence of caries.

Other galvanic couples used as medical devices are also prone to galvanic corrosion for example, the screw-plate complex for fracture fixation or the pedicular screw-rod system for posterolateral arthrodesis. Often different alloys are employed for different device components, based on their mechanical behavior (e.g. plates and screw made respectively of stainless steel and TiA6V). All orthopedic surgeons have documented examples with corrosion pictures, associated with surrounding tissue discoloring or even necrosis. Systemic increase in the level of released metallic ions may create mutagenicity or clastogenicity concerns. The mechanical resistance of the device is impaired through the corrosion process, even leading to implant rupture. In these examples, the intrinsic biocompatibility of each component is established according to conformity to specifications for implantable materials, but the clinical conditions of use create additional features that will impact the toxicological profile as well as the final performance of each device.

All these examples of failure are used to document the clinical concept of biocompatibility. Could these cases of failure be avoided? Perhaps not fully but at least some of them may have been prevented by not only fulfilling a matrix of biocompatibility requirements, but also by introducing into the biological risk evaluation all the actors of the interface, material scientists, designers, manufacturers and subcontractors, biologists, toxicologists and end users with an exact knowledge of the instructions for use according to patients' pathologies. Moreover, biocompatibility evaluation should always refer to the tissue, site and mode of clinical use, in order to be as predictive as possible of any possible biological harm.

2.4 Challenges in biocompatibility evaluation

ISO/EN Standards of the 10993/30993 series represent, theoretically, an appropriate level of biological assessment of MDs. Although considered to represent a biological safety assessment, the continued use of these standards

for more than 30 years now raises challenging concerns. Among these, the duration/conditions of extraction procedures, the interpretation of test results in this relatively young scientific and industrial model are discussed.

2.4.1 Extraction conditions

Several biological tests, for example, cytotoxicity, irritation, sensitization, systemic acute/chronic toxicity, genotoxicity, hemolysis and chemical characterization are performed on extracts of the device. Both polar and non-polar extracts are required to represent the broad class of extractibles, in surface/volume ratio largely exceeding clinical conditions, but the extraction duration is generally of 72–120 h. The ISO 10993-12 standard does not suggest any longer extraction period. Whilst 72–120 h may represent a sufficient duration for the extraction of leachables or process residues, these conditions will never cover all the potential H extractables from the material itself (plasticizers, residual monomers, polymerization initiators, degradation products, ions from metallic alloys, crosslinkers, stabilizers, preservatives, thermal treatment of Ni-Ti shape memory alloys, etc.) during the in-life conditions of a long-term implantable device.

This was the rationale used by some regulators to introduce the concept of exhaustive extraction for some biological evaluations (genotoxicity, sensitization). However, on the one hand not all regulatory agencies accepted these conditions, and on the other hand they may result in complete destruction of the material that would no longer be representative of clinical use. Nevertheless, there is a real issue that should be more widely discussed at the standardization level, since high-level biological risks, such as mutagenicity or toxicity, are stop-go tests. Up to now, they are performed on 72 h extracts that are not predictive of mid-term or long-term changes of the material. Alternate or additional conditions, based on chemical and clinical risk assessment, should be considered by regulatory bodies.

Rationale for the use of additional ‘non-standard’ extraction conditions should be based on chemical characterization of extracts obtained in a screening phase, with a panel of extraction conditions representative of the clinical use/risks, in order to justify and select the most appropriate validated conditions that will be submitted to biological evaluation.

2.4.2 Revisiting the design of sensitization tests

Macromolecules, such as collagen and latex, are well-known sensitizing agents, able to induce in humans both systemic acute type I immune response as well as delayed type IV hypersensitivity reactions. Neither macromolecule tested as extracts in the maximized guinea pig Magnusson and Kligman

test, designed to evaluate type IV reactions, induces any hypersensitivity reaction. However, when second induction includes an intradermal injection together with the topical application, and when using an intradermal challenge performed 48 h after the topical challenge, then extracts of both macromolecules induce a 80–100% type IV sensitization. For latex there is even a dose relationship between the incidence of sensitization in guinea pigs, with the amount of leachable proteins present in the extract.

This example emphasizes that a ‘false’ safety result can be associated with the non-availability of the reactants to the test system (the antigen-presenting cells of the dermis/profound epidermis) due, in this example, to high cutaneous impermeability to macromolecules when administered topically.

2.4.3 Interpretation of biocompatibility test results

Cytotoxicity tests, irritation assays, sensitization and hemolysis are biological tests where the standards define acceptance criteria or criteria for ranking the biological response. Threshold values for these tests are generally established based on long experience of device testing.

For systemic toxicological testing, acceptable safe values obtained for clinical, biochemical, hematological and histopathological parameters should not be biologically significantly different from control groups.

Similarly, the evaluation of the local tolerance is based on semi-quantitative scores of the components of the histological reaction, and threshold values are proposed by the ISO 10993-6 standard for the interpretation of the results. For blood compatibility complement activation, the use of both control material and reference materials already in clinical use for similar indications is recommended. However, no threshold acceptance criteria are established.

Similarly, genotoxicity evaluation is based on interpretation of results founded on 70 years of experience in chemical and drug genotoxicity evaluation. In all these tests, a positive control is used, which is one of the most potent genotoxic drugs. Threshold values generally do not exist or are fixed at twice the negative control values and test results are always compared with the positive drug compound. However, the potential genotoxic components of a device probably have metabolism, clearance, storage and elimination kinetics that are completely different from drugs. In addition to the use of extracts obtained in conditions already discussed in Section 2.4.1, the decision to rank a result as positive is based on threshold values established for chemical drugs. In several assays for devices in which a potentially harmful questionable component exist, a slight but regular increase of the genotoxic expression, as compared with control values, should be carefully assessed, whether metabolic activation is necessary or not. This suggestion may apply to both eukaryotic and prokaryotic assays.

In addition, due to extract dilutions in these test conditions, very low doses of material are generally tested and the final ratio exposure should always be considered when evaluating results. As an example, an extract of 6 cm²/mL (highest ratio of the standard) will expose 100 µL in 2.5 mL of the bacterial mutagenicity test system (Ames test). This means the equivalent 0.6 cm² in 2.5 mL, that is, a surface exposure in the Ames test system of 0.2 cm²/mL. The significance of such extract dilution remains to be addressed. Therefore, the biological evaluation of a 'discrete' effect, obtained under these conditions is highly recommended instead of a comparison with 'positive' controls that only validates the reactivity of the test system. The same comment applies to the micronucleus test where the extract injections 24 and 48 h before animal sacrifice are based on a drug metabolism kinetic but not on the potential of leachables to interact locally or systemically with liver, kidney, bone marrow, etc.

2.5 Conclusion

Science is the basis for developing standards. Biocompatibility standards are mandatory to assess the safety and performances associated with medical devices. Clearly, the risk analysis process, the appropriate knowledge of the chemical components of a device, its clinical conditions of use and clinical data evaluation, should be an essential part of the biological assessment, as recommended by the new ISO 10993-1 standard. As a consequence, the requirements for more precise protocols for chemical and biological evaluation, founded on adequate scientific rationale, should be considered by regulatory agencies and the MDs industry as a complementary step to improving the expertise in this newly developing area of MDs and to avoid future large scale public health issues. New materials, new indications, new conditions of use should be carefully reviewed by scientists, industrialists and regulatory bodies. Key features for their assessment remain that:

- All tests should be conducted in accordance with the Good Laboratory Practices.
- Tests should be performed in accordance with established internationally agreed methods but regulatory bodies should also consider scientifically sound and justified protocols, when appropriate, which could according to a risk assessment complement the 'standardized' evaluation protocols.
- Animal welfare considerations and particularly the reduction of the number of animals used are fundamental issues, thus permanent *in vitro* alternatives and chemical characterization/evaluation of leachables/extractables should be employed wherever possible with future refinement on safety margins and acceptance criteria for these tests.

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Biological safety evaluation planning of biomaterials

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Abstract: ISO 10993-1:2009, *Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process* states that this ‘document should serve to be the framework of a plan for a biological evaluation’. ISO 10993-1 indicates that such a plan ‘should minimize the number and exposure of test animals by giving preference to chemical constituent testing and *in vitro* models in situations where these methods yield equally relevant information to that obtained from *in vivo* models’. It stresses the fact that animal testing should not be used to uncover information that is already known. The document also states that biological safety evaluations ‘must be planned’ in advance. It is a working document of execution. It should document what should be done, when it should be done and why. Thus the overall value of the biological safety evaluation plan is that it defines the evaluation of the unknown so that a sufficient amount of information can be evaluated and risk can clearly be understood and characterized.

Key words: biological safety, biocompatibility, biocompatibility plan.

3.1 Introduction

ISO 10993-1:2009, *Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process* states that this ‘document should serve to be the framework of a plan for a biological evaluation’. This really does not represent a change in thinking in the document. It is just that, for the first time, ISO 10993-1 has spelled it out for us. The standard is just a framework, and the tables in the annex of the document are tools used to develop evaluation plans. They are not checklists of what must be done, because no checklist can satisfy the requirement of the standard.

3.2 The fundamentals of safety evaluation planning

ISO 10993-1 stresses the fact that animal testing should not be used to uncover information that is already known. The document also states that biological safety evaluations ‘must be planned’ in advance.

Most importantly, the biological safety evaluation plan defines how the firm will assure the safety of its product. An effective plan starts with material selection. The objective is to pick materials whose properties are most suitable for the performance of the device. Given those requirements, materials are characterized and qualified, so that the risk of an adverse biological effect arising from the use of that material is negligible. After qualifying the material, the component is qualified. We then examine the molding process – specifications such as molding cycle time, generations of regrind permitted, melt flow range and other parameters that could have an effect on the material and whether the processed material could change the safety profile of the material. Once completed, we have a tremendous amount of confidence in the safety of that component and material. We can now apply that information across a range of devices and uses.

Combining the information from the raw material supplier and final product test results assured device safety by mitigating the biological risk previously identified. This is exactly what the FDA desires.

Part of communicating the biological safety plan to team members is the definition of tasks, milestones and key events in the schedule of bringing this device to market or in advancing the project schedule. Gantt charts and timelines drive schedules. Yet most companies wait to communicate with the regulatory agencies, such as the FDA, until late in the process and, when they do, they are less than clear. The plan also can be used in preliminary discussions with these agencies, so they can better understand the firm's thought process. If the firm wants to work closely with FDA, they can use the test plan as an effective communication tool with the agency. Even if the company declines the opportunity to work with FDA early on, this evaluation plan is the perfect instrument for proper communication of the firm's rationale for assuring safety.

In addition, the plan acts as documentation of prevalent thought within the firm, its vendors and consultants.

Planning is a documented process. The process is of extreme importance, while the document is merely the end product of hours of research, deliberation, discussion and decision making. This chapter will focus on the planning process that culminates in decisions and action steps that allow us to complete our evaluation in the pre-clinical stage. The evaluation, however, is never quite complete but rather extends onto both the clinical stage of the product and during post-market activities. Those post-market activities include product changes, all of which must be addressed within the context of the original plan. This method is far more comprehensive than checking off a list of suggested tests. While it may be perceived as a 'relaxed' or 'shortcut' method of evaluation, it is actually far more thorough in its scope of assessment.

The biological safety process is a continual process that is dependent upon known information. As information changes, evaluation becomes

mandatory. The first stage of this evaluation is to understand the impact of the information.

3.3 Safety evaluation planning for biomaterials

In order to assure safety, one has to address risk. The checklist approach assumes that, given a list of discrete categories of body contact and duration, the risk to the patient never changes. That is like saying that the risk of driving from New York to Tampa is identical each time I do it. It does not take into account my vehicle, the time of day I am driving, the weather conditions, traffic, etc. Of course, this is absurd but this is what occurs when you truncate an incredible variety of medical devices into a handful of categories.

I do not mean to belittle the contribution that the original authors of the 1987 FDA Tripartite document made. It was they who first related biological safety to body contact and duration of use. This effort was actually brilliantly handled, given the fact that so many devices could be correctly characterized thereby allowing for proper application of the method.

Despite its drawbacks, the checklist approach has one great advantage. It standardizes and simplifies the process allowing regulators to easily judge how biological safety is being assured. In summary, it is easy to use, easy to teach and does not require much thinking. Only knowledge of materials and the process by which they are assembled, coupled with risk assessment, gives rise to information that is needed to construct the biological safety evaluation plan.

In order for a biological safety evaluation plan to be just as effective, it needs to convince the reader that the steps being taken to ensure safety adequately address the biological risks of product use, so that successful completion of these actions steps will give us the confidence that the product is biocompatible and that this biocompatibility can be confirmed clinically. What the plan should not be is a list of tests to be completed and a list of tests to be waived. Unfortunately, there are documents, including FDA's G95 Blue Book Memorandum, that indicate that the purpose of risk assessment and planning is to waive tests. This is simply not true. The purpose of the plan is to describe how a company will utilize limited resources (because indeed all resources are limited) to perform the best possible evaluation to assure biological safety. The plan should also be a pragmatic, 'hands-on' document. It should describe the necessary steps for implementation along with the acceptance criteria.

For example, a manufacturer makes an IV Catheter. The catheter material is the same material that the manufacturer uses to produce another short-term blood contact device, a guide wire. The production processes for both are similar in that neither impacts on biocompatibility. In order for the plan to be effective, it would have to deal with the pragmatic approach of leveraging testing and other information for the material as a guide wire. Merely to state that certain tests will be completed when indeed they were

conducted as part of another device is both confusing and misleading and will indeed lead to more regulatory questions. Stating very clearly what exactly will be accomplished is much more effective and better understood – both good reasons for initiating the plan to begin with.

It should be authored by someone who has the necessary education and/or training/experience to have knowledge of how the 10993 series should be applied and used. In addition, that person should have a firm knowledge of the device in question, its materials of construction, the manufacturing process and manufacturing materials, and studies that have already been conducted providing information about potential toxicological risk.

3.4 Developing and documenting plans

Prior to initiating a biological safety evaluation plan, a risk assessment should be conducted. In Annex I of *ANSI/AAMI/ISO 14971:2007, Medical devices – Application of risk management to medical devices*, it is stated that applying risk assessment to biological hazards involves an examination of a range of effects of potential biological hazards. These hazards could include short-term effects such as acute toxicity, irritation to the skin, eye and mucosal surfaces, hemolysis and thrombogenicity, as well as long-term or specific toxic effects such as subchronic and chronic toxic effects, sensitization, genotoxicity, carcinogenicity (tumorigenicity) and effects on reproduction including teratogenicity. These possible effects are evaluated in conjunction with intended use. Intended use drives the perception of device risk and also provides us with limitations as to where and how biological safety might be impacted.

The assessment begins by analyzing all potential biological hazards given the intended use of the product and the materials of composition. In reviewing the materials of composition, one should remember also to consider materials that are used during manufacturing but do not constitute part of the bill of materials of the device. Such ‘manufacturing materials’ may remain on the device in the form of small quantities of residuals.

The assessment begins with a collection of information regarding the product and the conduct of a literature search, in order to determine what is known about the device and what remains unknown. A prescribed plan for literature search must be documented. Appendix C of ISO 10993-1 gives us excellent guidance on how literature must be studied and how to assure that an unbiased assessment is conducted. If there is insufficient information to address a specific biological endpoint, then obtaining this unknown information should be targeted in the plan. This would be likely to result in conducting tests so that data can be generated and the evaluation can be completed. We must consider all research in literature but some literature is more valuable than others. Human data are often most applicable, but if

these data are occupational inhalation data, they may be less valuable than IV data on a rat when investigating a blood contact device such as an IV catheter. If we are looking for slight behavioral or adverse effects induced by a repeated dose study (these effects are generally reflected by a NOEL or NOAEL value), then a short-term LD₅₀ value would be of little use.

This is process is a cyclic one because the evaluation of biological safety is continual. Action is taken in an effort to reduce risk. The results of that action lead to re-evaluation. Changes in the product lead to re-evaluation, and finally the acquisition of new information can lead to further assessment. In reality, all three of these occur.

The plan documents the balance between known and unknown information and how such information impacts on the ultimate goal of demonstrating adequate safety when compared to the benefit of device use. What the plan should not be is a regurgitation of Annex A in ISO 10993-1. In order to understand this, one must understand that biological safety cannot be completely assured by the testing of one sample replicate. Consistency of materials and consistency of processing are mandatory prerequisites to evaluating biological safety. Without proven process validation and material qualification, it becomes impossible to evaluate the safety of a device because one cannot establish what the device truly is. Validation is documented proof that the device can be made as per documented specifications in a reproducible manner. Once reproducibility of product and process is established, then we can evaluate the product and be assured that our evaluation will impact all validated products.

A full understanding of the impact on biological safety is required. The purpose of the plan is to sort out the information that is needed to make such a judgment and acquire it.

3.5 Using safety evaluations

‘Paperwork’ is often a bureaucratic exercise that has little value. A ‘working document’ on the other hand has lasting value. The biological safety evaluation plan is the quintessential working document. It establishes the who, what, when, where and how of the evaluation sequence and documents it for historical reference so that future evaluations are consistently applied. It is a working document of execution. It should document what is to be done and why. It should also address when it is to be done.

Essentially, biological safety can be divided into four categories:

1. systemic toxicity,
2. local toxicity,
3. carcinogenicity,
4. special considerations, that is, blood contact, central nervous system (CNS) contact, etc.

Each of these is handled quite differently during planning. The plan often hinges on a risk assessment. The assessment hinges on known information. There is typically a large amount of information in the areas of systemic toxicity and carcinogenicity. There is often quite a bit documented on irritation as well, but there is less information on sensitization and histopathology upon implantation. Special considerations are even rarer.

Even if information is available, one has to consider the impact of the manufacturing process. This applies to both converting the material to the polymer you purchased, and processing and assembling the materials and components into the finished device. The risk assessment examines this information and more. The document characterizes the risk so that it is clearly understood what is already known and understood, as against that which is unknown. The more information that is not well understood, the greater the risk to the safety of the patient.

Thus the overall value of the biological safety evaluation plan is that it defines the evaluation of the unknown so that a sufficient amount of information can be evaluated and risk can clearly be understood and characterized. It documents the thought process behind the choices made in conducting testing, in choosing the way the test is conducted, and in waiving the conduct of certain tests that may be expected otherwise. For example, a polylactic acid (PLA) implant that has been well characterized with regard to both short-term and long-term toxicity may have little information with regard to skin or tissue contact. The easiest way to uncover this type of information is to perform an implant study to demonstrate that the device is safe with regard to local tissue contact. The length of the study would depend on the type of information that is discovered either through literature or other testing.

It also tends to limit any regulatory deficiencies to those that essentially disagree with your assessment rather than those that seem quite unordinary in terms of scope and magnitude. I like to look at this using the sports expression 'home field advantage.' This is to say that it is to your advantage to set the subject matter on which the regulatory debate will ensue. Your preparation of the assessment and the plan will have made you quite comfortable and knowledgeable in this area.

3.6 Conclusion

There is no doubt that this process is far superior to checking off a list of tests and submitting them to your reviewer. These test methods have withstood the test of time. There is a great deal of comfort and trust in the reliability of these tests. However, they are probably the most inadequate means of evaluation.

In conclusion, the next time your needs turn to biological safety do not initiate testing until you have documented a plan for ensuring biological safety.

3.7 Sources of further information and advice

ISO 10993-1:2009, Biological safety of medical devices – Part 1: Evaluation and testing within a risk management process.

Biomechanical and biochemical compatibility in innovative biomaterials

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Abstract: A wide range of materials have been used in medical devices that rebuild normal functions of a whole or a part of a living structure. The choices of materials have advanced from the bioinert, to the bioactive and the bioresponsive. The interfaces between man-made materials and biological materials are highly important for a diverse range of applications. The understanding of the structure, bonding and dynamics of the biointerfaces is a prerequisite for the design of new materials with improved biocompatibility.

In this chapter, the biomechanical and biochemical compatibilities are discussed with reference to the current concepts, the state-of-the-art development and future challenges. Examples are given from a new generation of metallic alloys, addressing the problems of ‘stress shielding’ of traditional metallic implant materials, to the development of self-assembled drug-deliverable coatings.

Key words: biomaterials, biocompatibility, biointerface, metal, polymer, ceramic, composite.

4.1 Introduction

A wide range of biomaterials, from metals, ceramics and polymers to composites, have been used in medical devices that perform, augment or replace a natural function of a whole or part of a living structure, such as contact lenses, dental implants, artificial skin, heart valves, breast implants, joint prostheses and bone plates, as shown in Table 4.1.

Materials for tissue repair and replacement date back to ancient Egypt, where metals were used for fracture fixation. Since then, our understanding of the interaction of biomaterials with the human body has been constantly improving. The choices of materials have advanced from bioinert, to bioactive and bioresponsive materials. The creation of new biomaterials has been targeted to repair and replace damaged or diseased human tissues through an iterative process, thus providing more physiologically compatible replacements. In this chapter, the biomechanical and biochemical compatibilities, the two key aspects of a successful new biomaterial, will be

Table 4.1 Applications of biomaterials

| Applications | Materials |
|------------------------------|--|
| Contact lens | Hydrogel |
| Middle ear implant | HAPEX™ |
| Dental implant | Titanium |
| Artificial skin | Collagen-GAG composite |
| Heart valve | Reprocessed tissue, stainless steel, carbon |
| Catheter | Silicon rubber, Teflon, polyurethane |
| Fracture fixation | Stainless steel |
| Bone grafts | Calcium phosphates, Bioglass® |
| Joint prosthesis (hip, knee) | Titanium, Ti-Al-V alloy, stainless steel, cobalt-chromium alloys, polyethylene |
| Bone cement | Polymethyl methacrylate |
| Breast implants | Silicone rubber, silicone gel |

GAG, glycosaminoglycan.

discussed with reference to the current concepts, state-of-the-art development and future challenges.

4.2 Selection of biomaterials

According to the type of tissue to be replaced in the body, biomaterials can be generally categorised into hard materials, used in bone replacement (e.g. dental and orthopaedic applications) or soft materials for cardiovascular (heart and blood vessels) and plastic surgery.

It seems that metals are the first group to be considered for hard tissue replacement, and polymers for soft tissue replacement. In fact, all three major groups of solids, metals, plastics and ceramics are represented among bone replacement materials for various applications, and new composites are emerging at fast pace to offer closely matched replacements.

In general, medical devices and prostheses are often made of more than one material; for example, hip replacement prosthesis mainly consists of a metal head coupled with an ultrahigh molecular weight polyethylene socket (Learmonth *et al.*, 2007). This couple, pioneered by Charnley, was the globally preferred articulation throughout the 1970s and 1980s.

However, the generation of micron and submicron particles of polyethylene (PE) wear debris triggers osteolysis from the cytochemical response, and leads to aseptic loosening, which has emerged as the major cause of failure of total hip replacement. Is there a solution to reduce PE and/or metallic wear debris? The wear debris seems unlikely to be eliminated from articulating surfaces in joint replacement currently; can we control their generation or make them more compatible with the body?

Recognition of the problems associated with PE resulted in an increase in the use of metal-on-metal implants, especially for younger patients

(<50 years of age). Although the volumetric wear of metal-on-metal bearings is substantially less than that with metal-on-PE, a far greater number of much smaller particles are produced. Increased concentrations of metal ions have been widely reported in the peripheral blood and urine of patients with metal-on-metal couples (Grubl *et al.*, 2006; Witzleb *et al.*, 2006). The changes of the materials used for the articulation will increasingly result in younger patients being exposed to higher amounts of metal ions and particles for extended periods. In the development of alternative new materials, these very complex biocompatibility issues need to be considered first, as metals are well known to produce complex biological actions with immunological, mutagenic and toxic effects.

Ceramic materials, such as alumina and zirconia, have superior corrosion resistance in comparison with metallic alloys, and have been considered as important alternatives to surgical metal alloys used in total hip prostheses and as tooth implants. High-purity alumina has high strength and fatigue resistance for the load-bearing requirement in the skeletal system due to a very small grain size (< 5 μm) with narrow size distribution. The main advantages of using ceramics over the traditional metal and polymer devices are lower wear rates at the articulating surfaces, and the release of very low concentrations of inert wear particles, which provides a solution to overcome the implant failure resulting from excessive wear rates.

Zirconia ceramic has higher fracture toughness and flexural strength, thus offering a promising prospect as an alternative material for bearing surfaces in joint replacement prosthesis. However, there are considerable concerns with its wear properties, strength degradation, and particularly the premature fracture of a batch of ceramic femoral heads has caused a Food and Drug Administration (FDA) recall. Therefore, a comprehensive understanding of structure and performance of materials used and intend for use in medical devices has offered insights into the development of new materials for enhancing life.

Polymeric materials have many diverse applications, from skin, muscle, heart valve, breast implant, tendon, cartilage and bone to drug delivery, such as PE acetabular cup, polyhydroxyethyl methacrylate (PMMA) bone cement, silicone breast implants, polyester (poly(glycolic acid) (PGA), poly(lactic acid) (PLA)) sutures.

Many polymeric biomaterials used in the construction of biomedical devices (e.g. catheters, tubes, breast implants) are derived from products originally developed for industrial use. These have been clinically demonstrated to be biocompatible at first and have saved millions of lives, but there are still some uncertainties concerning their long-term stability and safety.

The most critical problem with all synthetic biomaterials is adverse tissue reaction. The prevalent issues involving breast implants include capsular contracture, gel bleed, implant rupture, infection, autoimmune disease and

various forms of cancer (Puskas and Luebbers, 2012). The alleviation of these complications is necessary in the design of future implants.

4.3 Three generations of biomedical materials

Instead of focusing on the structure, properties and functions of implant materials, three generations of biomaterials have been classified by Hench and Polak (2002) according to the interaction between material and host tissue.

Biomaterial in the first generation (before the 1970s) is generally considered as a systematically and pharmacologically inert substance designed for implantation within or incorporation with living systems. The key characteristic is bioinertness: the material does not interact with body fluids or tissues; is stable in the human body; and avoids adverse tissue reactions. Material was considered biocompatible if it was non-toxic, non-carcinogenic and had adequate mechanical strength as well as fatigue life.

The emergence of Bioglass® in 1971 marked the beginning of a new era of biomaterials, the second generation of bioactive materials. It has been recognised that the reactivity of Bioglass® with the surrounding environment leads to developing direct bondings with hard as well as soft tissue (Hench *et al.*, 1971; Hench and Paschall, 1973). The formation of this interfacial bond, due to the biological activity (bioactivity) of the material, could enhance the lifetime of an implant (Hench, 1991).

Another advance in second-generation biomaterials is the development of resorbable biomaterials, which can be broken down and resorbed, and ultimately replaced by new regenerating tissues, such as tricalcium phosphate (TCP) bone graft, PLA and PGA fracture fixation plates and screws in orthopaedics. The interface problem can be resolved, as ultimately, implant will be replaced by natural tissue.

In comparison with living tissues, which can respond to changing physiological loads or biochemical stimuli, synthetic materials have the limitation of being unable to respond to biochemical stimuli or biomechanical changes, although there are advances from the bioinert first generation to the bioactive or resorbable second-generation biomaterials. To target the limitation of the existing materials, third-generation biomaterials are being designed to stimulate specific cellular responses at the molecular level (Hench and Polak, 2002). Bioresponsive materials are being created to promote or inhibit specific cell activities. By immobilising specific proteins, peptides and other biomolecules onto a material it is possible to mimic the extracellular matrix (ECM) environment and provide a multifunctional cell adhesive surface or elicit specific interactions with cell integrins and thereby direct cell proliferation, differentiation, and ECM production and organisation. Bioactive glasses were found to be able to activate genes that stimulate regeneration of living tissues (Xynos *et al.*, 2001). Bioactive materials release chemicals or

growth factors such as bone morphogenic protein (BMP) that activate the cells in contact with the stimuli. The cells produce additional growth factors that in turn stimulate multiple generations of growing cells to self-assemble into the required tissues.

4.4 State-of-the-art development

The advance of science and technology has led to considerable progress in developing a new generation of medical implants and devices with improved performance. The following section provides a brief overview of the current understanding of ideal candidates of medical materials, in terms of materials chemistry, mechanical properties and surface characteristics.

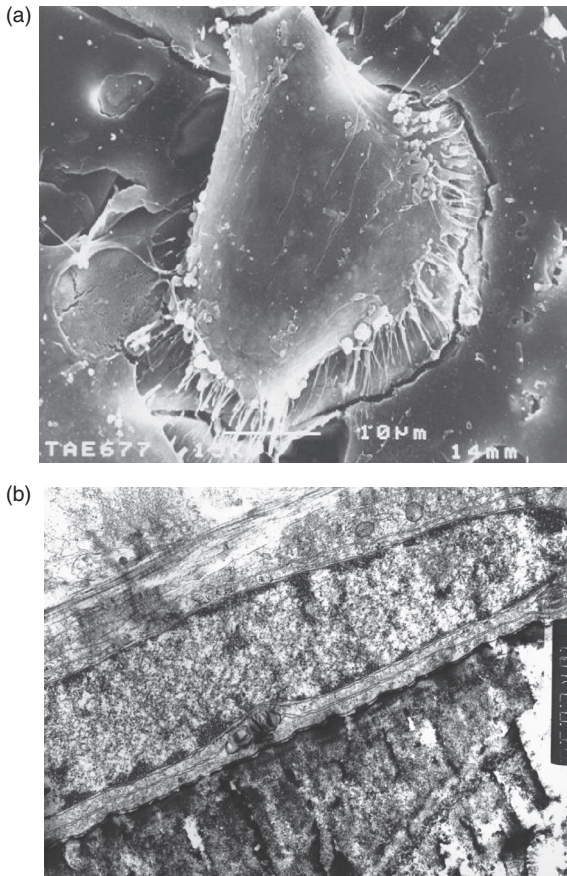
4.4.1 From biologically 'inactive' to 'active'

The use of materials in tissue reconstruction is to provide structural stability during healing or to replace impaired tissue. It is not surprising that initially the most important criterion in choosing these materials was chemical inertness. Depending on the degree of inertness of a material, the body's immunological response causes fibrous encapsulation of the implant of varying thickness. Generally, conventional implant materials have adequate mechanical strength and fatigue life, but are biologically inactive (nearly inert) and lack direct bonding with host tissue. Metallic implants have been widely used in major load-bearing applications, such as hip prostheses and dental implants due to their excellent mechanical properties, but osteoconductivity is generally lacking in metallic materials.

Bioactive materials are conceptually different from bioinert materials in that chemical reactivity is desirable and actually essential (Table 4.2). Bioactive materials, particularly, a series of bioactive ceramics, glasses and glass-ceramics, are capable of promoting the formation of bone at their surface and creating an interface which contributes to the functional longevity

Table 4.2 Different types of bonding at the materials–bone interface

| Materials | Biodynamics | Type of bonding |
|---|----------------------------|---|
| Stainless steel Polyethylene Silicone | Biotolerant | Formation of a thick layer of fibrous tissue |
| Alumina Zirconia Titanium | Bioinert | Formation of a thin layer of fibrous tissue |
| Hydroxyapatite, Bioglass® TCP, PGA/PLA | Bioactive Biodegradable | Formation of an interfacial bond Replacement of the surrounding tissue |



4.1 Attachment of human osteoblast (HOB) cells to Bioglass® particles in Bioglass®/polyethylene composite (a); a direction bonding of Bioglass® with HOB cells was formed at the interface (b).

of tissue. A direct bonding of Bioglass® with human osteoblast (HOB) cells is shown in Fig. 4.1.

Calcium phosphates are the major constituents of bone mineral. The most extensively used synthetic calcium phosphate ceramic for bone replacement is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, hydroxyapatite (HA), because of its chemical similarities to the inorganic component of bone and teeth.

The osteoconductive and osseointegrative nature of HA has made it a popular coating material for traditional metallic orthopaedic implants (de Groot *et al.*, 1987, 1998) and recently biodegradable magnesium alloys to control the degradation rate (Song *et al.*, 2010). HA-coated metallic implants promote better osseointegration (a) to direct tissue in growth or strong biological bonds with HA without the formation of concomitant fibrous

connective tissue, and (b) to inhibit the possible metal-ion release from the implant and protect the surrounding bone from potential corrosion products. Therefore, HA-coated metallic prostheses, which combine the osteoconductivity of HA and high strength of metal alloys, have been increasingly favoured by surgeons for younger patients seeking joint replacements. As reported in the Swedish register for hip joint replacement, increasing numbers of HA-coated prostheses have been implanted in patients in recent years. With the global ageing population, such a trend will continue strongly in the future.

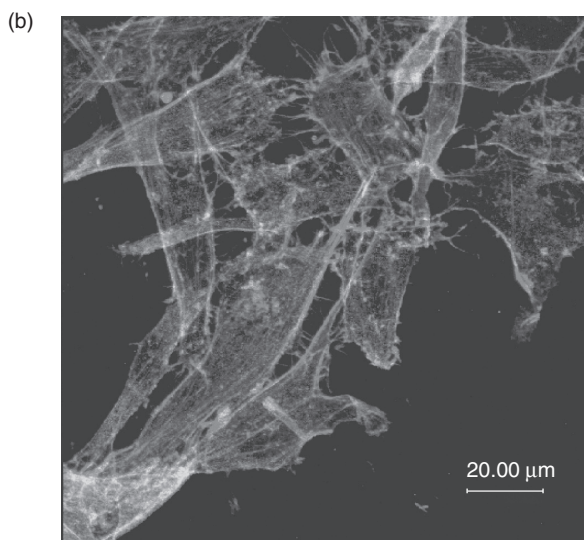
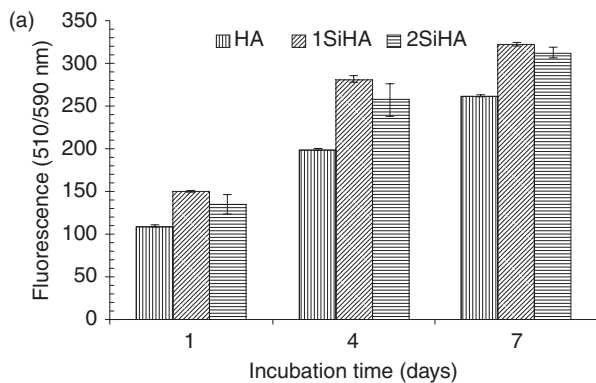
In addition to bioactive HA, it has been demonstrated recently that the *in vivo* bioactivity of HA can be significantly improved with the incorporation of silicate into the HA structure, silicon substituted hydroxyapatite (SiHA) (Hing *et al.*, 2006). With the addition of a small amount of silicon (~ 1 wt%) to HA, the bioactivity of SiHA has been increased (Fig. 4.2). SiHA has a greater rate of *in vivo* dissolution, in comparison with that of HA (Porter *et al.*, 2003) as well as a greater rate of bone apposition (Patel *et al.*, 2002, 2005).

The addition of silicon in SiHA provides an extra chemical cue to stimulate and enhance bone formation for new generation coatings that promote the attachment and proliferation of HOB cells (Huang *et al.*, 2005; Thian *et al.*, 2007). The enhanced bioactivity of SiHA is the result of the increase in availability of Si as well as the favourable topography from increased grain boundaries with decrease in the grain size. Therefore, SiHA is a highly attractive alternative to conventional HA in bone replacement.

4.4.2 Mechanical biocompatibility: matching of stiffness

Stainless steel was the material of choice in early hip replacement surgery, but with the advance in material-processing technology, stainless steel has been generally replaced by cobalt and titanium alloys (Navarro *et al.*, 2008). Despite concerns of corruptions of metals (Jacobs *et al.*, 1998, 2003), metallic alloys are the materials used in the major load-bearing applications. Titanium alloys (Ti-6Al-4V) have better corrosion resistance in comparison with stainless steel (316 and 316L) and cobalt-chromium-molybdenum (Co-Cr-Mo) alloys, and have been used extensively as artificial hip stems and bone fixation devices. Although these alloys display excellent mechanical properties in terms of strength and toughness, there is a huge mismatch between the elastic moduli of these alloys and that of cortical bone (Table 4.3), which is believed to be an underlying cause of the loosening of an artificial hip stem.

Ti alloys have a relatively low elastic modulus (e.g. 110 GPa) in comparison with Co-Cr alloys (230 GPa), but are still much higher than that of cortical bone (up to 30 GPa). One of the problems of metallic implants



4.2 (a) Comparison of the growth of HOB cells on HA and silicon substituted HA (SiHA) during 7 days of culture, a higher proliferation rate was found on SiHA. (b) Actin cytoskeleton and vinculin adhesion plaque of HOB cells attached on nanoSiHA coating by electrospraying.

in hip replacement, such as femoral stem prosthesis, is that implants take a considerable part of the body loading, which shields the bone from the necessary stresses required to maintain its strength, density and 'healthy' structure. This 'stress shielding' will lead to bone resorption, eventually implant loosening, and failure of the artificial hip. Moreover, toxic ions (e.g. V and Al) can be released into the body, and raise long-term biocompatibility concerns.

In the early 1980s, Bonfield *et al.* (1981) proposed that a match of the mechanical behaviour of an implant with the tissue to be replaced would

Table 4.3 A comparison of some mechanical properties of current implant materials with those of cortical bone

| Materials | Young's modulus (GPa) | Ultimate tensile strength (MPa) | Compression strength (MPa) | Critical stress intensity factor K_{IC} ($\text{MNm}^{-3/2}$) | Critical strain energy release rate G_{IC} (J m^{-2}) |
|--------------------|-----------------------|---------------------------------|----------------------------|---|--|
| Cortical bone | 7–30 | 50–150 | 130–240 | 2–12 | ~600–5000 |
| Cancellous bone | 0.1–1.5 | 1.5–38 | 1.9–13 | | |
| Co-Cr alloys | 230 | 900–1540 | | ~100 | ~50 000 |
| Stainless steel | 200 | 540–1000 | | ~100 | ~50 000 |
| Ti6Al4V | 110 | 900 | | ~80 | ~10 000 |
| Ti35Nb5Ta7Zr | 55 | 590 | | | |
| NiTi | 20–110 | 755–960 | | | |
| Mg alloys | 45 | 165–450 | 90–160 | | |
| Alumina | 365 | 6–55 | 4200 | ~3 | ~40 |
| Zirconia | 150–200 | | 1800–2000 | ~7 | |
| HA | 70–120 | 40–200 | 300–900 | ~1 | |
| Bioglass | 35 | 40–60 | | ~0.5 | |
| PMMA (bone cement) | 3.5 | 70 | | 1.5 | ~400 |
| PE | ~1 | ~30 | | | ~8000 |
| HA/PE | 1–9.9 | 20–90 | | | |
| DL-PGA | 1.9–2.4 | 29–35 | 29–35 | | |

Source: Bonfield (1987), Wang (2003), Navarro *et al.* (2008) and Witte *et al.* (2008).

eliminate the problem of stress shielding with conventional biomaterials. A new composite has been developed to meet the challenge of the longer lifetime required for the new generation implant materials, as the clinical success of a biomaterial demands the simultaneous achievements of a stable interface with connective tissue and a match of the mechanical behaviour of the implant with the tissue to be replaced (Bonfield, 1988). HA-reinforced PE composite, HAPLEXTM, has been commercialised as a successful middle ear implant. Since then, seeking ideal bone replacement materials are ongoing; a matched modulus of bone can be achieved through the composite approach (Wang, 2003). However, great efforts are still required to improve the strength.

From the compatibility of biomechanical aspects, the ideal material should not only have compatible stiffness, but also possess high strength and fatigue resistance. Therefore, considerable efforts have been devoted to enhance the yield strength and to reduce the modulus, such as new β - and (α/β) type titanium alloys (Liu *et al.*, 2004). Gum metal, with a general composition Ti–24(Ta + Nb + V)–(Zr, Hf)–O, is a multifunctional β -type titanium alloy with an ultra-low elastic modulus, high strength and superelastic-like deformability (Saito *et al.*, 2003), although the mechanisms for their unusual behaviour have yet to be clearly identified.

One of the key selection considerations in the formulation of new alloys is the elimination of toxic elements. To overcome the limitation of the conventional trial and error approach, the first-principles electronic structural calculations (discrete variational cluster method) have been used for the design of novel high-strength to low-modulus Ti alloys with much improved biochemical and biomechanical compatibilities, and thus offer theoretical guidance in practical structural design. Song *et al.* (1999, 2001) suggested that Zr, Nb, Mo, Hf or Ta may increase the strength and decrease the modulus of bcc Ti. To reduce the potential toxicity of implant, Nb, Mo, Hf and Ta are the most suitable elements for titanium alloys. These considerations dictate that the new generation biomedical titanium alloys developed mainly consist of Ti, Nb, Ta and Zr (Hao *et al.*, 2002, 2007; Li *et al.*, 2004; Chen *et al.*, 2009; Guo *et al.*, 2009).

Nanophase alumina (with grain size of 23 nm) has been synthesised to further improve the mechanical properties of alumina ceramics, the modulus of nanophase alumina decreased by 70% (Webster *et al.*, 1999), which is promising in consideration of mismatched properties with human cortical bone.

4.4.3 Multifunctional surface modification

HA coating on metallic implants by plasma spraying has achieved great clinical success. However, the high processing temperatures and poor coating integration with the metal surface are the major drawback for this well-established technology. A range of low temperature techniques have been merging as an alternative to coat the implant surfaces with bioactive HA (Radin *et al.*, 1997; Habibovic *et al.*, 2002; Liu *et al.*, 2002; Huang *et al.*, 2005; Leeuwenburgh *et al.*, 2005; Thian *et al.*, 2006), which can offer a number of advantages, such as incorporation of growth factors or antibiotic for enhanced biological response.

Inspired by nature, a new strategy has been developed for the functionalisation of the surfaces. In nature, living organisms, such as bones and egg-shell membranes, employ various acidic macromolecules to control the nucleation and growth of their mineral phase, such as polysaccharides with sulfate groups, proteins with carboxylic acids and phosphate groups.

Self-assembly monolayer (SAM) is one of the nanofabrication methods to create nanostructures with desired interactions between biomaterial and host tissue. The formation of SAM on the surfaces of metals and metal oxides has found many applications, such as in the immobilisation of biomolecules, protective coatings, friction and lubrication control, surface functionalisation, etc. (Campbell *et al.*, 1996; Sato *et al.*, 2000; Liu *et al.*, 2002; Majewski and Allidi, 2006; Toworfe *et al.*, 2006). SAM is able to tailor the

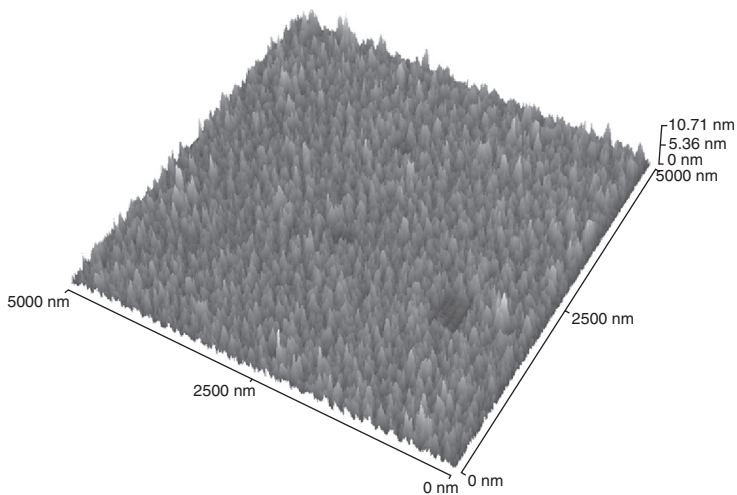
surface properties to facilitate the growth of HA for enhanced bioactivity. For example, alkylsilane SAM with sulfonic acid functional groups has been applied to the surface of Ti-Al6-V4 alloy to induce the nucleation of calcium phosphates (Bunker *et al.*, 1994).

One of the risk factors associated with the orthopaedic surgery is post-implantation bacterial infection. To overcome the limitations of the common systemic delivery of antibiotics, local delivery of drugs offers more advantages, such as effective therapies, decreased systemic toxicities and side effects, higher therapeutic drug concentrations in the relevant tissues and reduction in the necessary treatment duration.

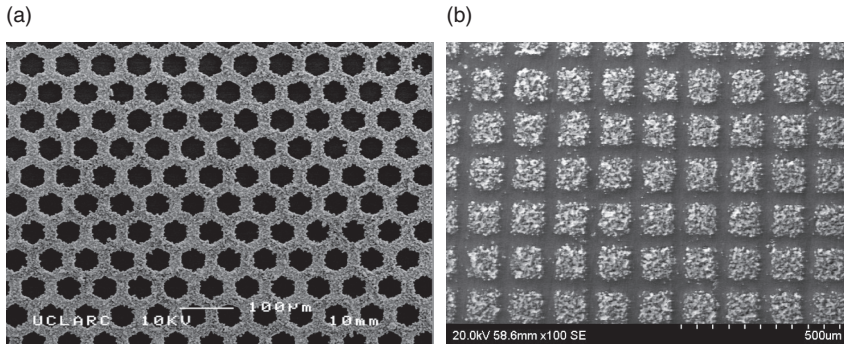
The multifunctional surface modification has been attempted by SAMs, where negatively charged end groups (i.e. $-\text{COO}^-$) were applied to accelerate the nucleation and growth of HA on the Ti surfaces, and neutral end groups (i.e. $-\text{CH}_3$) be applied to inhibit the nucleation and growth of HA but to immobilise other functional groups, such as antibiotics. Thus the implant surface can be tailor-made with dual or multiple functionalities (Campbell *et al.*, 2000; Ajami, 2010).

Ciprofloxacin (CFX) is one of the common antibiotics used in the treatment of post-implantation bacterial infection. It has one $-\text{COOH}$ and one $-\text{NH}$ head group. Immobilisation of HA/CFX on a Ti surface has been prepared based on hydrophobic-hydrophobic interactions of octadecyltrichlorosilane (OTS) based SAM (Fig. 4.3).

Microfabrication techniques have become increasingly popular to optimise the material surface for desirable interactions, such as mechanical



4.3 AFM images of OTS film by SAM on Ti surface (Ajami, 2010).



4.4 (a) Hexagonal and (b) square patterns of nanoHA byTAEA.

micropatterning, coating and chemical grafting (Castner and Ratner, 2002; Park *et al.*, 2007).

A desirable tissue response to implant materials can be achieved by enhancing the interaction between tissue and biomaterials. It is well known that cells recognise surface features and react to them, resulting in contact guidance (Curtis and Wilkinson, 1997). One of the key design parameters for medical implants is the surface topography, which has been found to provide a powerful set of signals for cells. There is increasing evidence that surface topography both on the micro- and nano-scale are important in determining the cell response to biomaterials (Gray, 1996; Dalby *et al.*, 2007). Recent efforts have been directed towards surface patterning in the design of new generation intelligent implants. The creation of micro- and nano-scaled surface topography of HA and SiHA has been attempted by electrohydrodynamic spraying and print-patterning with the aim of up-regulating cell activity (Huang *et al.*, 2004; Ahmad *et al.*, 2006; Li *et al.*, 2008, 2010; Thian *et al.*, 2008; Munir *et al.*, 2011), as shown in Fig. 4.4. Controlling cell direction, orientation and proliferation rates is of paramount importance in the success of an implant as it enables not only decreased implant fixation time, but also cells to grow preferentially in one area to strengthen fixation in desired areas. Therefore, the design of the implant surface is crucial to promote the acceptance of implants by surrounding tissue and ultimately, extending the functional service life of the implant.

4.4.4 Control of biodegradability

Both non-degradable and degradable materials have been used for tissue repair. When the mechanical stability is essential in the applications, non-degradable materials are used, such as Co-Cr alloy femoral stem, PE acetabular cup, PMMA bone cement and silicone elastomer breast implant.

Metallic alloys, such as stainless steels, are used as temporary implants in the form of plates, screws and pins for the repair of bone fracture. One drawback is that a secondary operation is required to remove the fixation once the tissues have healed. A biodegradable material, which dissolves completely afterwards, will provide a solution without the need of the second operation. Therefore, the current trend is favouring degradable materials, which initially serve as temporary scaffold for mechanical and biochemical support, and after the degradation the implant will be replaced by new regenerated tissue. It can provide a solution to the problems associated with 'foreign body' intrusion, although there are many challenges in providing such an 'ideal' solution, such as sufficient mechanical properties, suitable compositions and structures (for cellular infiltration and maintenance of cell phenotype), desirable degradation rates, easy surgical handling and implantation, and the reduced mismatch between the device and the surrounding native tissue.

PGA and PLA are the most popular biodegradable polymers (Gupta and Kumar, 2007), which allow hydrolytic degradation through de-esterification. Once degraded, glycolic acid and lactic acid can be removed by natural pathway. PGA and PLA sutures have been used for several decades (Dardik *et al.*, 1971). PGA can degrade in a few weeks; PLA is much more hydrophobic and degrades slowly, due to the additional methyl group. The degradation rate and mechanical properties can be tailored by creating copolymer of PGA and PLA, such as PGA-PLA bone screws (Athanasίου *et al.*, 1996). However, the acidic nature of both the glycolide and lactide monomer produced by the degradation changes the local pH, which may lead to adverse tissue response. The ability to support tissue regeneration needs to be considered carefully for biocompatibility, and the mechanical strength of PGA and PLA is still relatively lower for the major load-bearing applications.

Magnesium has a compressive yield strength (65–100 MPa), close to that of natural bone (130–180 MPa), and seems a more suitable candidate material for resorbable implant applications with a relatively high load-bearing requirement. It degrades in the physiological environment; the electrochemical reaction (corrosion) produces magnesium hydroxide and hydrogen gas. Magnesium hydroxide accumulates on the underlying magnesium matrix as a corrosion-protective layer in water, but when the chloride concentration increases in the corrosive environment, magnesium hydroxide starts to convert into highly soluble magnesium chloride. Severe pitting corrosion can be observed on magnesium alloys.

Alloying is one of the common methods used to enhance the degradation resistance of metallic materials, such as alloying with aluminium, zinc, calcium and rare-earth elements (Witte *et al.*, 2008). The reduction of the degradation rate of magnesium alloys will improve the degradation resistance and make it a biocompatible implant material.

TCP is a biodegradable bioceramic that dissolves in wet media and can be replaced by bone during implantation. To match the rate of resorption with that of the expected bone, tissue regeneration for a biodegradable material is a great challenge. When its solubility is higher than the rate of tissue regeneration, calcium phosphate will not be of much use in cavity filling. TCP with Ca/P ratio of 1.5 is more rapidly resorbed than HA. A mixture of HA and β -TCP, known as biphasic calcium phosphate (BCP), has been attempted as a bone substitute (Daculsi *et al.*, 2003). It has the advantage of tailor-making its chemical properties, such as varying the ratio of HA/ β -TCP. The higher the TCP content in BCP, the higher the dissolution rate. The resorption rate of BCP can then be monitored and controlled.

4.5 Future trends

A wide range of materials have been used in medical devices that rebuild normal functions of a whole or a part of a living structure. The choices of materials have advanced from the bioinert, to the bioactive and the bioreponsive. However, the current implant materials are still unable to outperform their native counterpart, the understanding of biocompatibility and biointerface of new materials and development of suitable evaluation methods will play key roles in the continued search of better replacement materials for tissue repair and regeneration.

4.5.1 Biocompatibility

The early biomaterials (e.g. in orthopaedic and dental surgery), are chemically inert which was considered as 'compatible' with the physiological environment. The latest understanding is that 'biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy' (Williams, 2008). The focus has shifted to achieve 'desired function' and provide enhanced performance. Nowadays biocompatibility is one of the key criteria for the clinical success of an implant or device.

A material that does not generate a toxic effect will no longer be considered as a sufficiently biocompatible implant material. It has to perform or integrate with host tissue appropriately. The biocompatibility of a material is related with a range of materials issues, such as chemistry, composition, micro/nano structure, morphology, crystallinity, porosity and surface

characteristics. All have impact on the performance of the materials, for example, ion release profile and ion toxicity in addition to corrosion properties for metallic material; and degradation profile, leachables, additives, catalysts and contaminants for polymeric material.

Medical grades of stainless steel, cobalt-chrome alloys and titanium alloys have been used to avoid hostile reactions from the human body. If corrosion resistance is the key consideration for the biocompatibility of the metallic implants, how can we improve the crucial property in the development of a new material?

To improve long-term stability of an implant, implant materials need to be either bioactive or resorbable. The bioactive material surface can provide sites for heterogeneous nucleation and crystallisation of a hydroxyl-carbonate apatite layer that induce cellular reactions leading to the formation of new bone. Resorbable biomaterials on the other hand, will be replaced by regenerating tissues, leaving no discernible difference between the implant and the body tissues.

The new generation biomaterial aims to stimulate specific cellular responses at the molecular level through more biologically-based methods. The ability of a material to promote desirable interactions will determine the outcome of the biocompatibility test as well as the potential of the new materials.

4.5.2 Biodegradability

The relationship between biodegradable materials and the host responses is highly complex, the degradation process or the corrosion products can induce local inflammation and the products of inflammation can, in turn, enhance the degradation process. Therefore, the biological responses of biodegradable metals need to be fully understood. For example, the high degradation rate of Mg at physiological pH poses a major challenges for its application, as the mechanical integrity of magnesium implants during service may be adversely affected (Staiger *et al.*, 2006).

The controlled corrosion of implants seems a challenging field. The oral administration of acid nutrients or diluted hydrochloric acid has been suggested to prevent the fast degradation of Mg. Formation of abscess cavities and fast disintegration of Mg seemed undesirable for fracture fixation, while extensive fibrous tissue formation in the resorbed Mg areas, which impregnated with hydrogen gas bubbles, was found to stop local bleeding (Witte, 2010).

Recently, biodegradable magnesium implants have attracted interest in cardiovascular applications (stents) and musculoskeletal (osteosynthesis) applications. However, the human body system must be able to remove the extra Mg ions at a high rate, which may not be sustainable over a relatively short period of time.

4.5.3 Biointerface

For all the applications of biomaterials, a desirable interface between the material and body is one of the critical criteria for the potential success of the materials. Cells in the body do not react directly with the materials. Instead, there is a mediator between them, mainly biomolecules, especially proteins. Proteins react with the surface and pass messages onto the living cells, which will then react accordingly. To understand fully the change of proteins at the interface is crucial in designing biointerfaces for improved proliferation and differentiation of relevant living cells.

The human body considers biomaterial as an 'intruder'. This causes a series of chemical and physical reactions to the interface. The whole process starts with the adsorption of proteins existing in blood plasma such as albumin and fibrinectin on the material surface. This adsorbed layer provides the template for cells to act. The proteins adsorbed on the surface of the biomaterial will undergo conformation and orientation change. Many factors can affect the adsorption of proteins, including the enzymatic influences, the host hematological properties and the physiochemical properties of the material.

The physicochemical properties of implant materials, such as surface energy and charge, hydrophilicity or hydrophobicity, tend to affect biological response by influencing protein absorption and cell attachment. Therefore, materials' surface properties can have a great impact in the control of biocompatibility of new materials. The nature and development of a stable interface between an implanted materials and host tissue is critical for the clinical success of the implant.

4.5.4 Nanomaterials

Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. When particles are reduced to the nanometer scale, the huge increase in the surface area of nanoparticles will result in significant changes in the properties from their corresponding bulk counterpart materials. The resultant increases in chemical reactivity also raise the question of the biocompatibility or cytotoxicity of nanomaterials.

In the development of a bioactive composite for tissue replacement, optimising the structure and biological response of the filler particles is essential as the structure of fillers (size, composition, and crystallinity) can influence cell viability/activity. Incorporation of nano-sized HA was found to be able to improve the mechanical properties and protein absorption of the composite (Wang *et al.*, 2002; Wei and Ma, 2005); the results have encouraged

various attempts to formulate new bioactive nanocomposites. These nanocomposites closely resemble the bone structure, but their properties, particularly the releasing of nanoparticles to the biological systems, require thorough investigation for the potential clinical applications (Huang *et al.*, 2008).

Due to the ability of pathogenic microorganisms to develop resistance to virtually all known antibiotics, the interest in metal nanoparticles and their oxides as antimicrobials has gathered pace (Allaker and Ren, 2008). There is huge potential for applying nanomaterials to enhancing the functions of medical devices. Recent study showed that substituted HA nanoparticles were able to inhibit the growth of four bacterial strains, including multi-antibiotic resistant EMRSA 15 and EMRSA 16 'superbugs', which is the first step in the development of multifunctional dental and orthopaedic prostheses (Huang *et al.*, 2011).

Nanoparticles are being used to improve the effectiveness of the overall drug delivery, as diagnostic and therapeutic agents to detect and treat human diseases. The biologically active ingredient may be adsorbed or chemically bonded to the particles' surface or incorporated into the particles by dissolving, entrapping or encapsulation. Human exposure to nanoparticles seems inevitable, therefore it is important to the understanding the properties of nanoparticles and their effect on the body, which is crucial to avoid undesirable toxicity before the potential clinical application (Lewinski *et al.*, 2008).

4.5.5 Smart responsive materials

Smart materials have huge potentials for medical applications. Nickel-titanium (NiTi) alloy with 55 wt% of Ni and 45 wt% of Ti is known as NITINOL. The unique shape memory properties, which allow the spontaneous recovery of shape after being subjected to deformation higher than their elastic limit, have led to applications in tailored compressive fixation of bone fragments, anchoring of implants and dentures to the living tissues, and intravascular devices (Liu *et al.*, 2004). Shape memory technologies have shown great promise for cardiovascular stents; a small stent can be inserted along a vein or artery and then expanded to prop it open.

pH-sensitive or pH-responsive polymers are being explored in controlled drug delivery due to their responsive ability of varying dimension by swelling or collapsing with the change of the pH of their environment. For instance, thermoresponsive polymers have sparked various biomedical applications including drug delivery, tissue engineering (Bikram and West, 2008). A targeted drug delivery is the key to achieve a satisfactory therapeutic outcome, otherwise the drug may end up in a different part of the

body, and toxic to healthy tissues. ‘Smart’ pH-sensitive nanoliposomes are being developed that are stable at physiological pH but in the acidic environment they undergo degradation and release the active substance (Liu *et al.*, 2011).

4.5.6 *In vitro* evaluation

In vitro cell culture, allowing the biological assessment of materials at a cellular level, is becoming increasingly useful for the evaluation of a new biomaterial, as it is possible to test any toxic effect on human cells, which is an important first step in screening materials before *in vivo* evaluation and further clinical trials.

For building up a comprehensive knowledge of cellular responses of new materials, both direct and indirect testing and evaluations are required (ISO standard 10993). In direct testing, the test materials are brought into direct contact with the cells. The damage to cell membrane as a result of the contact is the sign of cytotoxic effects. In indirect testing, cytotoxicity is measured by the exposure of the cells to the aqueous extract of the test materials for given time periods. Any potential toxic leachable into the surrounding environment/medium can therefore be detected.

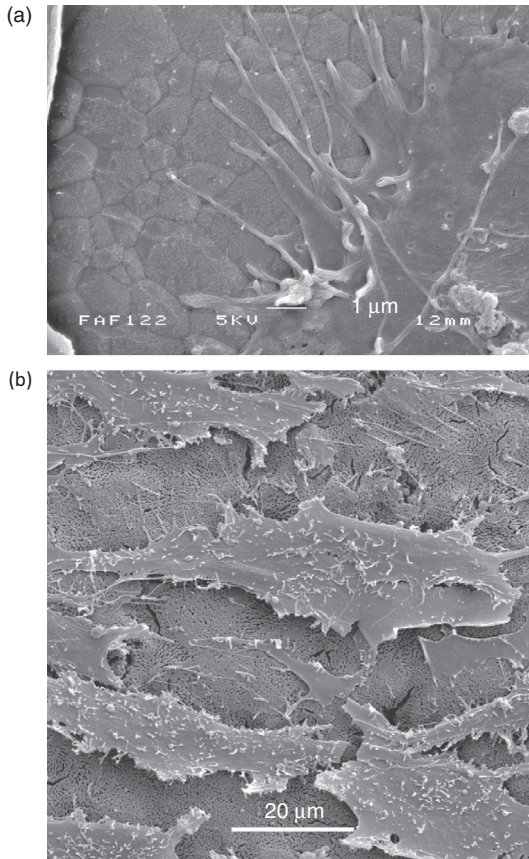
For bone replacement material, the osteoconductive potential is related to the biological responses from HOB cells, and much attention has been directed to the study of the behaviours of HOB cells, particularly the attachment, adhesion and spreading at the initial stage of cell/material interactions, as the quality of this interaction will influence subsequent cell proliferation and differentiation (Fig. 4.5).

However, tissues and organs are not made up of a single type of cell, and the interactions of different cells play essential roles in physiological functions of cells. Therefore, various co-culture models, more closely resembling the potential interaction of the implant with tissues in the host body, have been developed, such as endothelial cell/osteoblast, osteoclast/osteoblast, chondrocytes/osteoblast (Jiang *et al.*, 2005; Spence *et al.*, 2009; Unger *et al.*, 2011).

4.6 Conclusion

New materials/devices are emerging to meet the requirement for human wellbeing in the twenty-first century, such as new generation metallic alloys to overcome the problems of ‘stress shielding’ of traditional metallic implant materials, and development of a self-assembly drug-deliverable coatings, antimicrobial nanoparticles to fight infection and particularly ‘superbug’.

With the advance of materials science and engineering, particularly nanotechnology, new materials are emerging at a rapid pace. The design



4.5 The attachment of HOB on (a) sintered SiHA and (b) nanoHA coating by electrospraying.

of new materials to meet the new challenges and the understanding of the interactions between biomaterial and host tissue is highly important. Great attention needs to be focused at the interface between new materials and biological host, which is key for the potential successful clinical application of new materials.

Implant materials or devices interact with the biological environment through a biointerface, while the surface structure of the biomaterials determines the interfacial properties. The interfaces between man-made materials and biological matters are highly important for a diverse range of applications, from traditional medical implants to novel protein chips. The understanding of the structure, bonding and dynamics at the interfaces, particularly at the atomic level, is a prerequisite for the design of new materials with improved biocompatibility and biofunctionality for short term as well as long-term success of medical devices.

Bioactive and bioresponsive materials are a developing trend for the future. Undoubtedly, these materials will release a relative high level of 'foreign species' or products, at least in the short term. Their potential effects on tissue, particularly at the genetic level, need to be clearly identified and could be the major challenges to future success.

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Material and chemical characterization for the biological evaluation of medical device biocompatibility

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Abstract: The importance of materials to the medical device industry cannot be over emphasized. Along with increased demand for medical grade materials, the medical device industry has renewed its awareness and concern for the safety of materials used. An essential part of establishing biological safety is the implementation of chemical and material characterization testing.

Chemistry is often called the central science because of its role in connecting the physical sciences, which include chemistry, with the life sciences and applied sciences such as medicine and engineering. This role of chemistry as the central science is very important to medical device evaluations and is noticeably evident in the ISO 10993 approach to the biological safety evaluation of medical devices and biomaterials. Parts 18 and 19 of the 10993 series of standards are receiving even more emphasis, as they become an integral part of the biological evaluation of medical devices. An important step in the process is that of characterizing the material and identification of chemicals that can migrate or extract from the polymer components. Such basic information is critical to understanding the biological response and toxicological risk of the device since adverse effects caused by materials are generally chemical effects.

In recent years there has been a great deal of discussion of the merits of chemical and material characterization. Considering the intrinsic worth of chemistry and materials characterization to biocompatibility, it seems very likely that chemical characterization will receive even more attention in the future.

Key words: material and chemical characterization, chemistry, extractables, biological response.

5.1 Introduction

Currently there is a great deal of emphasis on the use of chemical and material characterization to aid in the evaluation of medical device biocompatibility. It may not be obvious to many in the medical device industry why chemical and material characterization is so important and why it is an essential part of biological evaluation for medical devices. International

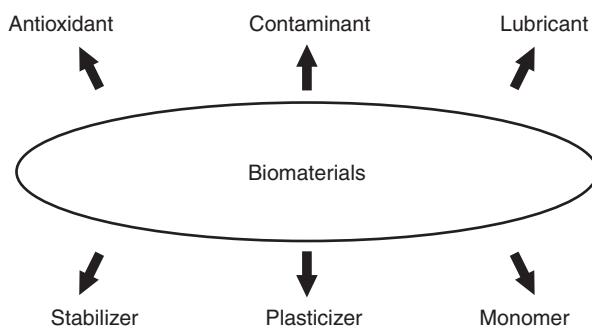
Organization for Standardization (ISO) harmonized standards clearly link the two together. ISO 10993, parts 18 and 19 are receiving even more emphasis, as they become an integral part of the biological evaluation of biomaterials and medical devices as described in the ISO 10993 series of standards.¹ These international standards have been and continue to be a major driving force behind the biological safety evaluation of biomaterials and medical devices.

With the changes to ISO 10993-1 in 2009, there is even more emphasis placed on chemical and material characterization testing within a risk management process, as part of the overall evaluation and development of each medical device. The biological evaluation of any material or medical device shall form part of a structured biological evaluation program within a risk management process in accordance with ISO 14971, the risk management document for the entire medical device industry. It is clear that the focus internationally in the assurance of biological safety must start with chemical and material characterization along with an assessment of toxicological risks as prescribed in ISO 10993-1, ISO10993-18, ISO 10993-17 and ISO 14971. Clearly, the process presented in these ISO standards and described in this chapter will aid in the selection of optimal materials and control the uniformity of those materials throughout the lifetime of the device.

5.2 Background

Establishing biocompatibility of medical devices and their materials is of key importance in assuring product safety. Central to the issue of biocompatibility is the role of chemistry and materials characterization. It is important to understand the material's composition as well as its physicochemical properties. Chemistry is often called the central science because of its role in connecting the physical sciences,² which include chemistry, with the life sciences and applied sciences such as medicine and engineering. This role of chemistry as the central science is very important to medical device evaluations and is noticeably evident in the ISO 10993 approach to the biological safety evaluation of medical devices and biomaterials. It is imperative to understand a material's chemical composition as well as the ability of chemical constituents and additives to migrate or leach into patient body fluids or tissues.

Another important consideration, however, is that the product or material have the necessary properties to carry out its proposed function. To evaluate these two fundamentally important issues, it will be necessary to perform material and chemical characterization on materials intended to be used in the manufacture and production of medical devices.



5.1 Polymeric biomaterials are composed of mixtures of chemicals, some of which are bound to the polymer backbone or into the material matrix while others are free to migrate into the surrounding environment. The identities and abundance of these chemicals determine a material's biocompatibility.

Adverse effects caused by medical device materials are generally chemical effects, produced by material components, contaminants or breakdown products that find their way from a device into a patient, causing a biological effect. Figure 5.1 shows the various types of chemicals that can migrate from polymers.

Presently, the biological evaluation of medical devices is governed by the set of standards developed by ISO and known as ISO 10993¹ or, in the United States, by the Food and Drug Administration (FDA) Blue Book Memorandum #G95-1,³ which is a modification of ISO 10993-1. This ISO document has recently been updated (2009) and establishes the need for toxicological risk assessment, chemical and material characterization prior to initiating any biological testing. At the heart of these international standard changes is a strong emphasis on chemical and material characterization for ensuring biocompatibility. This heightened awareness of the safety of medical devices has taken on an international character, in large part because of the convergence of FDA regulations and ISO standards.⁴ At the heart of these changes is the renewed awareness of the importance of chemical and material characterization as a necessary part of biological safety of medical devices.

The characterization of medical device materials is clearly identified by ISO 10993-1 as one of the first steps in the overall biological evaluation. Because of the importance of material and chemical characterization to biological evaluation, ISO 10993-18 was developed as a separate standard on the subject. Part 18 covers the requirements for providing information about the chemical composition of materials and devices, the potential release of leachable substances, and the predictive biological characterization of devices. Another standard, ISO 10993-19, entitled, 'Biological

evaluation of medical devices – part 19: Physicochemical, mechanical, morphological, and topographical characterization of materials,’ has also been developed. As the name indicates, the standard will address physical, mechanical and morphological characteristics of materials and final devices.

While it may not seem obvious to some why chemical and material characterization is an essential part of the biological evaluation of biomaterials and medical devices, ISO 10993 standards clearly link them. Understanding the principles presented in ISO 10993-1, 10993-18 and 10993-19 is imperative, and implementing them is an essential part of an overall biological evaluation program.

The goal of chemical and material characterization is to identify and quantify the chemical constituents and physical mechanical properties to help establish biological safety. ISO 10993 standards are intended to be implemented within a risk management process that combines the review and evaluation of existing data from all sources with, when necessary, the selection and application of additional tests.

Materials characterization forms the basis for understanding the composition of a medical device material and its potential to have an adverse biological effect when the device is put into clinical use. Material selection and risk analysis are integral components of the design process for medical devices and play critical roles in evaluating biological safety. Just as important as the testing, the biological safety evaluation plan should be designed and performed to demonstrate the achievement of specific criteria for safety. Materials characterization also serves as a means to ensure standardization of materials from one lot of devices to the next. Clearly, the process presented in ISO 10993-1, 10993-18 and 10993-17 and described in this chapter will aid in the selection of optimal materials and control the uniformity of those materials throughout the lifetime of the device. If appropriate care is taken to minimize critical controlling factors, *in vitro* material testing can frequently predict accurately the clinical outcome. Material characterization, which is a major component of the biological safety evaluation plan, is also an essential part of the regulatory process.

Identification of the constituents of a material intended for use in the manufacture of a medical device enables the intrinsic toxicity of the device to be investigated. Any of the constituents of a material or processing aids used in the manufacture of a medical device are potentially bioavailable. However, in order to estimate the risk of this occurring, it is necessary to obtain information demonstrating to what extent these chemical ingredients are available during intended use. This can be estimated from extraction tests on the device or material. In lieu of performing these tests, any biological hazards that could arise from the use of the material or manufacturing process are identified and the quantity of the chemical used is assumed to be entirely

available to the patient. In this manner a dose–response determination can be estimated from a perspective of worst-case.

Material selection and risk analysis are integral components of the design process for medical devices. The selection of materials plays a crucial role in evaluating the biological safety and, when approached in a systematic way, allows the collection of data.

The evaluation as a part of risk management includes identification of all hazards and the estimation of associated risk. A major component in hazard identification is material characterization. The following steps can be identified:

- define and characterize each material, including suitable alternative materials;
- identify hazards in materials, additives, processing and cleaning aids;
- estimate exposure (total or clinically available amounts);
- review toxicology and other biological safety data (published and available).

Risk analysis, the process of identifying the specific hazards and assessing their significance, begins with identification and characterization of the patient-contacting materials and components of the device. The evaluation starts with the assessment of the physical and chemical characteristics of the materials, history of clinical use or human exposure data, and any existing toxicology and other biological safety data on product and component materials, breakdown products and metabolites. To support biological safety, materials testing should be carried out on material samples that have been processed, including sterilization, in equivalent ways to the materials included in the final device.

The risk management approach emphasizes that conducting animal testing for risk evaluation should only be considered after all alternative courses of action (review of prior knowledge, chemical characterization, *in vitro* evaluations) have been established. Several clauses and subclauses in ISO 10993-1 ask the user to conduct chemical characterization of the device undergoing biological evaluation. This chapter will discuss what these ISO standards require and give some examples of what qualitative and quantitative tests can be used to satisfy the requirements.

5.3 Requirements of ISO 10993

Material characterization is designed to evaluate many different properties of medical device materials. ISO 10993-1 defines six different categories of properties that are to be evaluated. It states in section 4.1 (1) ‘that; in the selection of materials to be used in device manufacture, the first

consideration should be fitness for purpose having regard to the characteristics and properties of the material, which includes chemical, toxicological, physical, electrical, morphological and mechanical properties.' This section of the standard raises two essentially important issues. Is the material safe and does it have the necessary physical and mechanical properties for its proposed function? In other words, is the material biocompatible? To further emphasize these required tests, ISO 10993-18 (2) states: 'consideration of the chemical characterization of the materials from which a medical device is made is a necessary first step in assessing the biological safety of the device.' Part 18 makes it clear that this testing is necessary and required, but which tests are to be performed is not so clear.

To address the two basic questions about safety and function, chemical characterization is required to evaluate potentially leachable chemicals and their bioavailability, while mechanical/physical characterization will address functionality and safety. Morphological characterization will examine the surface of materials in an effort to explain or predict material interaction at the device–host (tissue) interface.

A variety of techniques are available to perform chemical and materials characterization. The tests may be carried out directly on material samples or on material extracts prepared under specified conditions. These tests, which have evolved over many years, are relevant, sensitive, rapid and inexpensive, yet provide extremely valuable information to establish material safety and biocompatibility.⁵ The extent to which a material needs to be characterized depends upon the type of material, the end use of the device, and the function of the material within the device. The more critical the role of the device and the more important the properties of its materials are to device performance, the more detailed the characterization program should be. Table 5.1 lists the various categories of devices defined in ISO 10993-18 and the proposed degree of characterization necessary.

Migration of material components, contaminants or breakdown products into a patient can have biological effects. Leaching of these same chemicals can adversely affect device functionality by altering its physical and mechanical properties.

Prior to performing any of the chemical or physical tests described below, it is very important to have precise information on the manufacture and production of the material. If the material is polymeric, then information on the synthesis of the polymer itself is important. For polymeric biomaterials, important information includes the following: (1) a description of the monomers used in the polymerization, (2) solvents used in the synthesis, and (3) special additives that have been added during production of the material. For devices with more critical functions or prolonged tissue contact, information about the sterilization process is important. Knowledge of degradation processes that the device material may encounter in its

Table 5.1 Degree of chemical and materials characterization based on tissue contact and duration

| Nature of contact and tissue type | Duration of contact | Types of devices | Degree of characterization |
|--|---------------------|---|----------------------------|
| Surface – Skin – Mucous membranes | Limited | Exam gloves, tape, blood pressure cuff Dental dams, endoscopes | Minimal |
| External communication – Blood, indirect | Prolonged | Dialysis, cardiopulmonary bypass | Intermediate |
| Implant – Blood, direct – Tissue contact | Permanent | Shunts or grafts Orthopedic implants | High |

intended function may be useful in identifying potential degradation products that may be released from the device. If adequate detailed information exists, the characterization program may be conducted partially or solely as a paper exercise.

In the absence of such details, appropriate analytical techniques must be applied to a material to yield compositional data. Sufficient information must be obtained to identify all toxic hazards arising from the chemical components of the material and sent for risk assessment, which will be discussed later in this chapter. When qualitative analysis alone has not provided sufficient data for a toxicological risk analysis to be completed, ISO 10993-18 requires quantitative chemical analysis be performed, documented and then sent for risk assessment. Completion of a characterization program requires the close collaboration of analytical chemists and toxicological risk assessors. The chemists provide the necessary qualitative and quantitative data that the risk assessors use to determine device safety. The flow of information between the chemists and the toxicologists is what establishes the process of materials characterization as an integral part of risk assessment and establishing biocompatibility.

5.4 Characterization of materials

Materials characterization is a crucial first step in the biological evaluation process.^{1,4} Material characterization is designed to evaluate many different properties of medical device materials. The extent to which a material needs to be characterized depends upon the type of material, the end use of the device, and the function of the material within the device. The more critical the role of the device and the more important the properties of its materials are to device performance, the more detailed the characterization program should be.

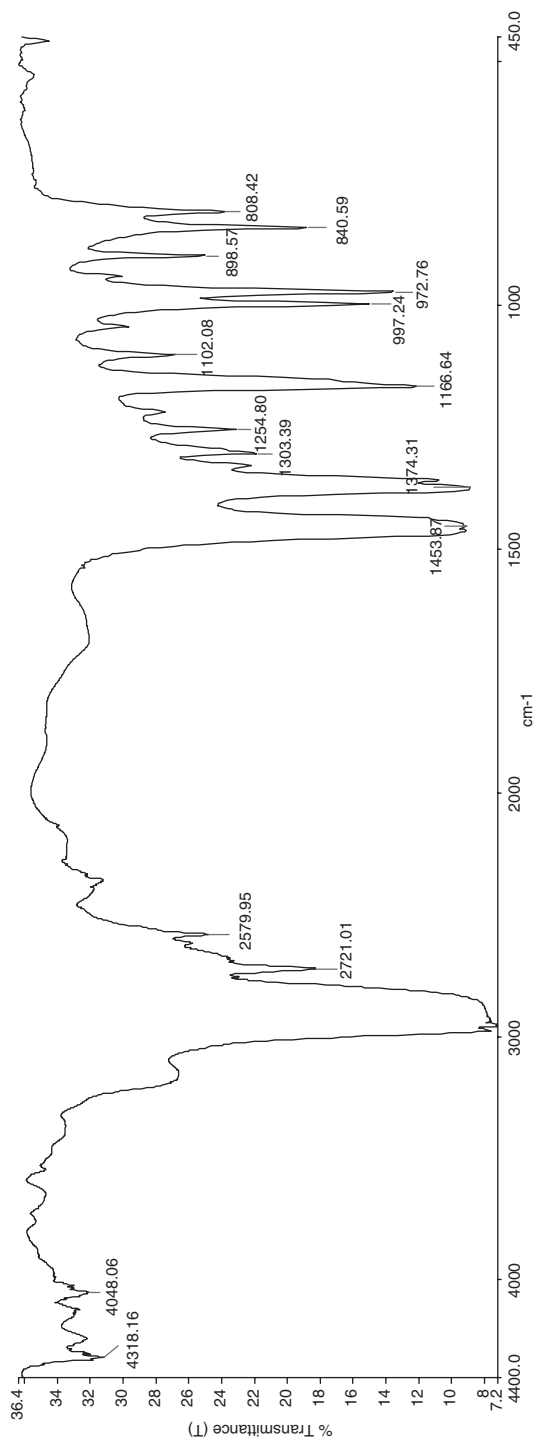
Infrared (IR) analysis is used extensively to fingerprint polymeric materials and should be a part of all polymeric biomaterials characterization programs. Since the IR spectrum of a chemical compound or biomaterial is perhaps its most characteristic physical property, IR analysis finds extensive application in fingerprinting or identifying materials.⁴ In this test, IR energy is passed through a thin film of material and the amount of energy absorbed at various wavelengths is measured.⁶ The result is a chart of wavelength versus absorption that is characteristic of the material (Fig. 5.2). By matching the IR spectrum of an unknown material with that of a known material, proof of identity can be established within the limits of the method. Reference spectra for common materials are commercially available, or may be accumulated through testing.

IR spectroscopy and especially Raman spectroscopy can be used to identify inorganic as well as organic structures. Techniques such as energy dispersive X-ray analysis or X-ray diffraction (XRD) analysis can be used to establish the type of ceramic or metallic material and some of its chemical properties. XRD is very important in identifying crystal structure and in crystalline phase analysis of ceramic materials. Of course, nuclear magnetic resonance (NMR) is one of the most powerful tools for molecular structure determination.

Thermal analyses are also useful for fingerprinting materials. These techniques measure the response of a material, generally a polymer, to controlled heating processes.^{4,7} In thermal gravimetric analysis (TGA), a plot of weight change is made as a material is heated at a known rate. In differential thermal analysis (DTA) and differential scanning calorimetry (DSC), an unknown sample and a reference sample are heated with the aid of a programming device and the temperature difference between the two is measured.⁷ Testing also can be conducted to determine the unique melting point, degree of crystallinity, and glass transition temperature (T_g) of a polymer. Both DTA and DSC techniques can be used to investigate the thermal properties of materials, including polymers, biological materials, inorganics and amorphous metal alloys. DSC results offer rapid measures of critical thermal characteristics such as polymer melting point or T_g . The particularities of these transitions, in addition to identifying characteristics unique to each polymer, can provide information about a material's phase structure, thermal history and purity.

The density of a solid is a conveniently measurable property that is frequently useful as a means of following physical changes in a sample, as an indication of uniformity among samples, and a means of identification.⁵ Changes in density of a plastic material may herald a change in crystallinity, loss of plasticizer, absorption of solvents, change in porosity and even changes in composition (proportions of resins, pigments or fillers).

Molecular weight is one of the most fundamental properties of any molecule. Almost all physical properties of polymers (synthetic or natural) systematically change as the molecular weight is altered. Unlike pure



5.2 IR analysis provides a rapid, effective means of identifying a polymeric material and of comparing samples to ensure consistency. The X-axis represents the wavenumber or frequencies of internal vibrations of the molecule in the IR region of the electromagnetic spectrum.

substances of small molecules, polymer samples have a range of molecular weights. For this reason, there is no such quantity as ‘the molecular weight.’ Instead, there are certain average molecular weights or molecular weight distributions (MWD). The most common analytical tool for measuring MWD is gel permeation chromatography (GPC), where the polymer molecules in a dilute solution are separated according to their hydrodynamic volume when forced through a column of microporous gel particles. Subtle changes in MWD can affect processing properties such as viscosity and cure rates. Mechanical properties, such as tensile and impact strength, elastic modulus, hardness and bond strength, can also vary with changes in MWD.⁴ GPC can be used to qualify incoming resins or device components as part of a materials qualification program, track lot-to-lot variability and evaluate product stability as part of a stability program or after radiation exposure.

The significant physical properties of a material can be identified with various test instruments. For example, stress/strain relationships such as tension, compression, shear and flexure are determined with a mechanical testing apparatus.⁵ Mechanical testing that measures stress/strain relationships is used to characterize a material’s behavior and performance. Stress/strain behavior greatly depends upon temperature, strain rates, and environmental conditions. These parameters must be understood and controlled during the testing process. The strength of materials is expressed by the ultimate values of stress, which can be found by loading the sample until fatigue. Loading can be tension, compression, or shear.

Material hardness is determined by means of a durometer that measures the extent to which the material can be compressed. It is measured for plastics, elastomers and metals. Surface properties, which are especially important for some specific categories of devices, such as those that contact blood, can often be observed directly using light, scanning electron microscopy (SEM), and atomic force microscopy (AFM).

5.5 Chemical characterization of extracts

The extent of characterization required depends on what pre-clinical and clinical safety and toxicological data exist and on the nature and duration of body contact with the medical device. As a minimum, it should address the constituent chemicals of the device and possible residual process aids or additives used in its manufacture.⁵ The identity and quantity of novel materials and chemicals present should be established or measured.

Medical device materials present a unique challenge to chemists and toxicologists, whose experiments usually involve chemical substances that can be delivered to a biological test system or ultimately to a patient. The preparation of fluid extracts of device materials is the most appropriate technique

to provide samples for determining the presence and toxicological reactivity of possible chemical leachables.⁴

The purpose of extraction of a medical device, with regard to chemical characterization, is to provide a suitable test sample to demonstrate the hazard potential (hazard identification) of the leachables and for use in conducting human health risk assessments of the leachables. If extracts of the device are prepared, the extraction vehicle and conditions of extraction used shall be appropriate to the nature and use of the final product as well as to the predictability (such as test purpose, rationale, sensitivity, etc.) of the test method. Extraction conditions and application of the extract to test systems, therefore, ideally shall reflect not only actual conditions of use of the products but also the purpose and predictability of the tests.

Extractions should be carried out at temperatures that are high enough to maximize the amount of extractable substances. However, extraction conditions should not cause deformation or degradation of the sample. Sample preparation is an essential part of every solvent-based extraction procedure. While many sample types can be efficiently extracted without any pretreatment, other samples may require some manipulation for an efficient extraction to occur. Spell and Eddy studied the extraction of additives from polypropylene at room temperature and found that required extraction time varied linearly with polymer density and decreased with increasing particle size.⁸

Extraction is a complex process influenced by time, temperature, surface-area-to-volume ratio, extraction medium and the phase equilibrium of the material. The phase equilibrium of a material controls the relative amounts of amorphous and crystalline phases present. For the amorphous phase, T_g dictates the polymer chain mobility and the diffusion rate in the phase. Usually the diffusion rate is considerably higher above the T_g compared with that below. The diffusion rate is lowest in the crystalline phase.

The two main factors in liquid–solid extractions are solubility of extractants in the solvent and rate of mass transfer out of the matrix (solid material). The mass transfer from the polymer is by diffusion from the bulk polymer to the surface, where dissolution in the extraction fluid can occur. Diffusion in polymers is a slow process, but it has been shown that increasing the temperature will exponentially increase the diffusion rate, which will lead to a higher extraction rate.⁹ However, increasing the temperature can also cause the polymer to undergo a transition from a glassy to a rubbery form at the T_g . The diffusion in the rubbery form is much faster than the crystalline glassy form. Therefore, a sharp rise in extraction rate would be expected at the T_g .

The extraction vehicle or fluid can also have an effect on the diffusion of low molecular weight chemicals from the bulk polymer that is attributed to swelling.¹⁰ This phenomenon, attributed to swelling of the polymer matrix by the absorbed solvent, improves the extraction rate by enhancing diffusion in the swollen polymer.

The extraction conditions should not alter the phase equilibrium of the material. Phase alteration may affect the amount and type of extractables. The effects of higher temperatures or other conditions on extraction kinetics and the identity of the extractant(s) should be considered carefully if exhaustive extraction is used. For example, there are a few concerns in using elevated temperatures:¹

- a) the energy of the increased temperature may cause increased cross-linking of a polymer and therefore decrease the amount of free monomer that is available to migrate from the polymer;
- b) the increased temperature could cause degradant materials to form that are not typically found in the finished device under use conditions;
- c) the increased temperature could cause the disappearance of a leachable material typically found in the finished device.

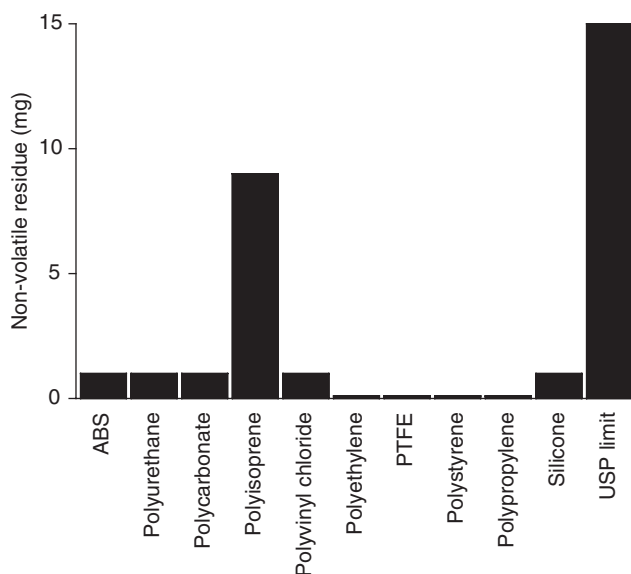
Since one of the limiting steps in extraction is diffusion to the surface of the polymer, the particle size or film thickness is extremely important. In some cases, grinding of the polymer is often a necessary step in the analysis. An exception to this is the extraction of thin films and foams, for which the shortest dimension is small.⁹

To further illustrate the complexity of the extraction process, the nature of the extractant affects extraction in both solubility and diffusion limiting cases. The larger the molecule the slower the diffusion in the polymer, and hence the slower the extraction process. High molecular weight compounds tend to be less soluble, and hence solubility will also limit the extraction more for large molecules.⁹

ISO 10993 parts 12 and 18 instructs that extractions differ for various test purposes:

- a) Exaggerated extraction is appropriate for hazard identification.
- b) Simulated-use extraction is applicable for generation of a safety factor for use in human health risk assessments.
- c) Exhaustive extraction is applicable for the assessment of the safety of an implant device and to estimate the upper limits of the chemicals that could be released to the patient.

Some potential extractables from medical device materials are water soluble, while others are soluble only in non-polar environments. For materials that will contact body tissues, extraction activity in both polar and non-polar environments is relevant. The *United States Pharmacopeia* (USP) includes physicochemical tests based on water and isopropanol extracts that are particularly useful in defining materials as rich or poor in extractables.¹¹ The tests categorize a specific material's extractables in general terms, such as non-volatile residue, residue on ignition, buffering capacity,

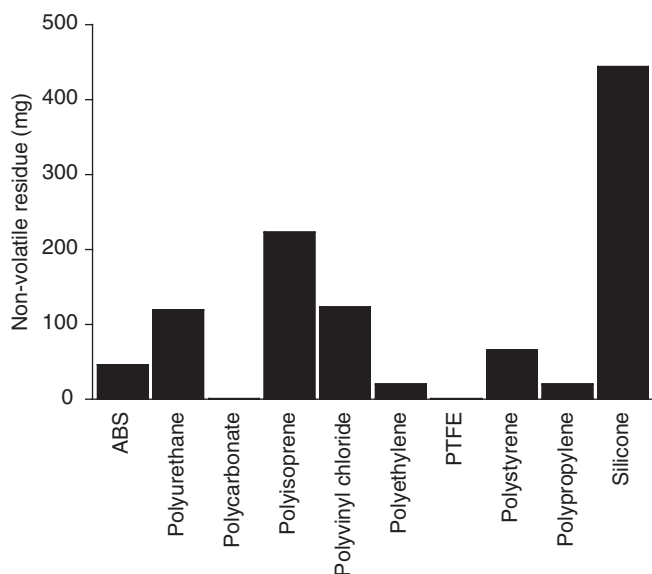


5.3 NVR results of aqueous extraction physicochemical testing on polymers commonly used in medical devices. Typically device materials contain very few water-soluble extractables. With reference to this test, USP limits can be used to establish specifications for raw materials. ABS, acrylonitrile butadiene styrene; PTFE, polytetrafluoroethylene.

heavy-metals content, ultraviolet absorption, and turbidity. The USP physicochemical tests for total extractables (non-volatile residue) should be a part of all characterization programs regardless of the criticality of the device or its function. The aqueous non-volatile residue is designed to determine the presence of water-soluble substances without regard to their identity. Figure 5.3 shows the results of aqueous extractions of polymers commonly used in medical devices. Typically device materials contain very few water-soluble extractables and do not exceed the USP limit of 15 mg non-volatile residue.

Determination of non-aqueous extractables should also be a part of all characterization programs. Typically alcohol, either ethanol or isopropanol, is a more aggressive extraction fluid and most device materials show measurable amounts of non-volatile residue. Figure 5.4 shows the results of alcohol extractions of commonly used medical device polymers.

Devices that fall into the more critical category of use require exhaustive extraction followed by analytical methods and instrumentation to identify and quantitate extracted chemicals. Exhaustive extractions are generally recommended for evaluation of materials intended to be implanted into the body. It is defined by ISO 10993-18 as a process that can extract 90% or more of the total available chemical species, including additives, processing



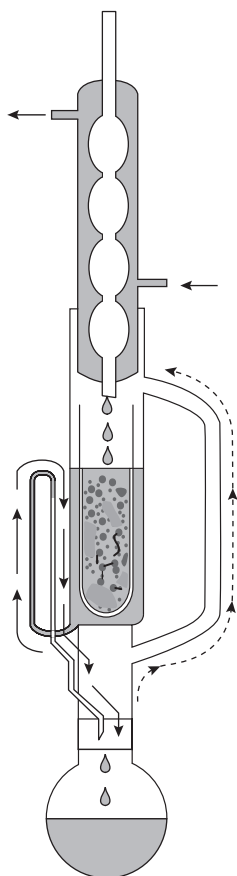
5.4 NVR results of non-aqueous extraction physicochemical testing on polymers commonly used in medical devices. Most materials used for devices show measurable amounts of extractables when tested with IPA. No USP limits exist to establish acceptable levels of extractables for materials tested by this method.

aids, sterilization residues as well as starting monomers. ISO 10993-12 provides the following definition:

Extraction until the amount of leachable material in a subsequent extraction is less than 10% of that detected in the initial extraction, or until there is no analytically significant increase in the cumulative leachable material levels detected.

There are numerous methods that can be used to extract low molecular weight substances from polymers. Examples include Soxhlet extractions, liquid–solid extractions, supercritical fluid extractions and microwave-assisted extraction. Soxhlet extractions will generally extract all additives, but extraction times can be long and labor intensive. A typical Soxhlet extraction setup is shown in Fig. 5.5.

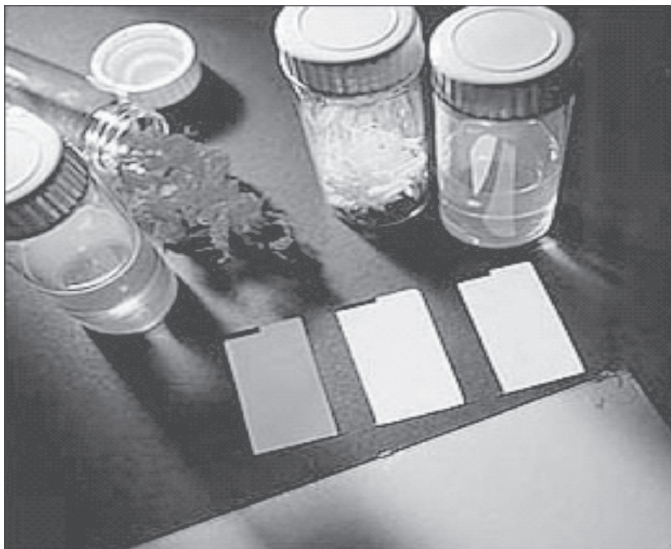
Liquid–solid extractions involve the intimate contact between a solid material, usually finely divided, and a solvent that has an optimum solubility for the analyte(s) of interest and minimal or no solubility for the material. For more intimate contact with the solvent and the material, additional sample preparation techniques such as subdividing into very small pieces may be required. ISO 10993-12 states that ‘Materials shall



5.5 Typical Soxhlet extraction setup.

be cut into small pieces before extraction to enhance submersion in the extract media except where otherwise inappropriate. For polymers, pieces approximately 10 mm × 50 mm or 5 mm × 25 mm are appropriate.’ Figure 5.6 shows samples prepared in this way for extraction.

Once the polymer has been extracted, the extracts must be analyzed both qualitatively and quantitatively to assess extracted chemical substances. There is no single analytical technique that could cover the identification of all potential extractables. Although there are specific analytical methods for particular additives, incorporating all these methods into a study such as this is neither practical nor necessary. Rather, a number of generic methods are used which are able to detect and identify a very board range of polymer additives. These techniques include gas and liquid chromatography with and without mass spectrometry, headspace gas chromatography, gravimetric and IR analysis using Fourier Transformed Infrared Spectroscopy (FTIR),



5.6 Extraction using materials cut into small pieces to enhance the extraction process.

inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma with mass spectrometry (ICP with MS).

The gravimetric and infrared analysis of the extract provides a generic quantification and identification of the major portion of the extract. The targeted species of this analytical method includes high molecular weight species, fillers, mineral oil, lubricants, plasticizers, silicone oil, and mold release agents. The gravimetric residue often comprises oligomeric material derived from the polymer⁶ due to degradation rather than extracted low molecular weight chemical additives and processing aids. IR analysis of residues containing such degradation fragments can be extremely valuable in establishing the identity and source of the oligomers.

Gas/liquid and high performance liquid chromatography (GLC and HPLC, respectively) are powerful analytical tools that can separate and quantitate volatile and semi-volatile chemicals.¹² Gas chromatography is applicable to the components of high to medium volatility and liquid chromatography to extractables of low volatility (sometimes referred to as non-volatile substances).

For materials characterization, these techniques can be used with extracts from, or in some cases solutions of, materials. Chromatography can produce qualitative, fingerprint-like information or, with appropriate standards, can be used to identify and quantitate specific chemical components. IR

analysis also can be used to fingerprint and identify the chemicals in an extract from a material, and mass spectroscopy methods can provide identification of specific molecular structures. Atomic absorption spectroscopy (AAS) can determine the amount of specific metals present in a material or its extract, while ICP spectrometry permits simultaneous determination of all the periodic table elements with a lower limit of detectability in the parts-per-billion range.¹³

5.6 Using chemical and material characterization to demonstrate equivalency

After performing a complete and comprehensive characterization study, it is reasonable to conclude that the characterization is appropriate throughout the life of the device. An unfortunate reality is that raw materials used in the manufacture of medical devices are frequently modified. When changes are made, two questions must be considered:

- Does the change invalidate the previous material characterization?
- How can the effect of this change on the material and, more importantly, on the device, be determined?

It should be noted that, based on a product's regulatory status, other testing also will be needed. For example, a premarket notification 510(k) submission to the USFDA for a medical device would require a different and probably less extensive level of testing than one covered by a premarket approval (PMA) application.

As mentioned earlier, ISO 10993-18 plays a pivotal role in the selection process, but the document can also help in dealing with material changes. The focus of this guidance document is on the chemical characterization of materials as an essential part of the overall biological safety of a device. It can also be used to judge chemical and toxicological equivalence. According to ISO 10993-18, equivalency is established when the composition and extractables profiles of the proposed (new) material is equivalent to a clinically established (original) material. Material equivalency refers to the sampling process for a subset of testing to confirm equivalent mechanical, physical and chemical properties for a particular material or one undergoing minor changes.

5.6.1 Change control and material equivalency

A material equivalency program must be developed as part of a company's change control process to evaluate material changes. One good model uses

a subset of chemical characterization testing. First, a screening procedure determines whether the material change produces a discernible change in the material's properties. That procedure also establishes the acceptance-testing criteria. If the screening study indicates that no change is detectable, the assessment is complete. A conclusion that the materials are equivalent can be drawn. If a discernible change is noted in the screening study, then the conclusion is that the materials are not equivalent. From there, the change is investigated in detail to determine its effect on material safety and performance.

Other options are available for determining the effect of a material change. One option would be to do nothing and hope for the best, or, at the other extreme, to repeat the chemical characterization of the material in its entirety. The first option is clearly unacceptable. The second option is not only costly and time-consuming, but is also impractical. A defined screening procedure is the best scenario for determining the effect of any changes.

It is worth noting that, in the course of such a procedure, a manufacturer may also find it useful to perform other screening. Other reviews that might be considered are evaluations of raw materials, methods of manufacture, and comments and data from the raw-material supplier. In addition, the effects of aging, the device's final assembly, and lab testing may enter the equation. However, such tests are beyond the scope of this chapter.

5.6.2 Screen testing

To confirm equivalent mechanical, physical and chemical properties for a particular material or one undergoing minor changes, a few select tests must be performed. These tests categorize specific material extractables in general terms, such as non-volatile residue (NVR), residue on ignition, buffering capacity, heavy-metals content, ultraviolet absorption, and turbidity.

5.6.3 USP physicochemical tests

The USP physicochemical tests for total extractable NVR should be a part of all characterization programs, regardless of how critical the device or its function is. The amount and properties of extractables are evaluated using methods described in monographs 381 and 661 of the current USP.¹¹ Collectively, these tests provide insight into the general chemical nature and amount of extracted substances.

Extracts of both the original and the new material must be done using comparable conditions. Model solvents typically used in the chemical characterization of the original material include purified water (PW) and isopropyl alcohol (IPA) for devices that are skin contacting and externally

communicating. PW, IPA and hexane are often selected for materials used in permanent implants. The USP physicochemical tests based on PW and IPA extracts are particularly useful in defining materials as rich or poor in extractables. The aqueous NVR test is designed to determine the presence of water-soluble substances without regard to their identity. Typically, device materials contain few water-soluble extractables and do not exceed the USP limit of 15 mg of NVR.

5.6.4 Infrared analysis

Used extensively to fingerprint materials, IR analysis should be a part of all characterization programs. In this test, IR energy is passed through a thin film of material, and the amount of energy absorbed at various wavelengths is measured. The result is a chart of wavelength versus absorption that is characteristic of the material (see Fig. 5.2). By matching the IR spectrum of an unknown material with that of a known material, proof of identity can be established within the limits of the test method. IR is also used to identify extract residues obtained in USP-model solvent extractions.

5.6.5 Thermal analysis

Also useful for fingerprinting materials are thermal analyses. In thermal gravimetric analysis, a plot of weight change is made as a material is heated at a known rate. In DTA and DSC, an unknown sample and a reference sample are heated with the aid of a programming device. It measures temperature difference between the two samples. Testing can also be conducted to determine the unique melting point, degree of crystallinity, and Tg of a polymer. This method helps establish the purity of both the new and original materials.

5.6.6 Gel permeation chromatography

Molecular weight is one of the most fundamental properties of any molecule. Almost all physical properties of polymers, synthetic or natural, systematically change as the molecular weight is altered. Unlike pure substances of small molecules, polymer samples have a range of molecular weights. For this reason, polymers do not have one quantity that is their molecular weight. Instead, there are certain average molecular weights, or MWDs. The most common analytical tool for measuring MWD is GPC. In this test, the polymer molecules in a dilute solution are separated according to their hydrodynamic volume when forced through a column of microporous gel particles. Subtle changes in MWD can affect processing properties such as viscosity and cure rates. Mechanical properties such as tensile and impact

strength, elastic modulus, hardness and bond strength can also vary with changes in MWD. This analytical tool can be used to qualify incoming resins or device components as part of a materials qualification program. It can also be used to track lot-to-lot variability or to monitor product stability periodically or after radiation exposure.

5.6.7 Cytotoxicity test

An *in vitro* biocompatibility test for cytotoxicity is described in ISO 10993-5. That test can evaluate the presence of any adverse biological effects from extracted chemicals. A minimum essential medium (MEM) extract is evaluated to assess the biological safety of extracted chemicals from the test articles. The extract is examined using a sensitive *in vitro* method to determine whether leachables extracted from the material would cause cytotoxicity or cell death. The results thereby give 'predictive evidence of material biocompatibility.'¹⁴

5.6.8 Hemolysis test

American Society for Testing and Materials (ASTM) F756, a standardized ASTM test method, can be used for determining the hemolytic potential of a device or material. The *in vitro* hemolysis test involves a quantitative measurement of plasma hemoglobin. Device materials are extracted in saline. They are then evaluated to determine whether the presence of any leachable chemicals from the test article could cause *in vitro* red blood cell hemolysis. This study is based on the requirements of ISO 10993, Part 4, 'Selection of Tests for Interactions with Blood.'

Using these few tests, it is possible to evaluate and confirm equivalence with respect to mechanical, physical, chemical and toxicological properties for any proposed material. It is important to remember that the level of testing must be adjusted to address the criticality of the end use of the device. Therefore, it is reasonable to conclude that testing performed for a surface device would be the least intensive, while an externally communicating or implant device would require more evaluation. Table 5.2 shows suggested tests per device category and can be used as a general guideline for test method and protocol development.

5.7 Acceptance criteria for equivalency

All measurements have some variability. The trick is to be able to tell when an experimental value is within normal variation or is outside of the ordinary. Control charts are designed to make this distinction possible. As long

Table 5.2 Suggested tests per device category for equivalency assessment

| Device category | Test procedures | | | FTIR | DSC | GPC | Cyto-toxicity | Hemolysis |
|--|-----------------------|-----|--------|------|-----|-----|---------------|-----------|
| | Physicochemical tests | | | | | | | |
| | PW | IPA | Hexane | | | | | |
| Surface devices | Y | Y | N | Y | N | N | Y | N |
| External communicating | | | | | | | | |
| – Bone/tissue | Y | Y | N | Y | Y | Y | Y | N |
| – Blood contact (circulating and direct contact) | Y | Y | N | Y | Y | Y | Y | Y |
| Implant devices | Y | Y | Y | Y | Y | Y | Y | N |

Y = yes (recommended); N = no (not recommended).

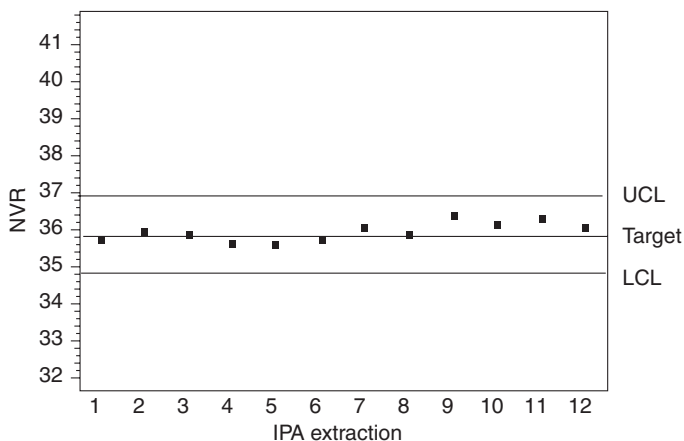
as all points lie inside the upper and lower control limits, the measured value is presumed to be normal or a common cause of variation.

When a data point (experimental variable or test) falls outside the limits of the control chart, it is necessary to identify a reason for the variation. Test values that fall outside limits are labeled not equivalent and further chemical characterization tests are needed to determine the effect of the change. This approach can also be used as a raw-material quality control test. It is designed to detect large variations or undesirably high or low properties.

Choosing the right control chart is essential when using this tool, since a data point can be quantitative (variable data) or qualitative (attribute data). FTIR spectroscopy and cytotoxicity and hemolysis tests result in qualitative data and have pass–fail criteria applied to them. If any test articles fail or do not meet the specified limit, then the material is considered not equivalent. Other tests are quantitative and yield numeric values. For those types of quantitative tests, using a control chart to evaluate the results is extremely valuable. A typical control chart constructed by plotting NVR versus IPA sample extractions is shown in Fig. 5.7.

The upper and lower control limits are established by calculating the mean and standard deviation of the test results. Then control limits are set to the standard deviation of the mean. This approach can be used on any experimental or test result and can be plotted as shown.

If the various test results indicate that there are no discernible changes, then no further testing is required and the test sample material can be qualified as equivalent. If there are discernible or significant changes noted, then the material is judged not equivalent. The material is either rejected or subjected to additional tests to further investigate the change and its potential overall biological or toxicological significance.



5.7 Documentation of results using control chart. UCL, upper control limit; LCL, lower control limit.

ISO 10993-18 states that sufficient qualitative information shall be obtained to allow a comparison to determine whether a material is equivalent to that used in a device with the same clinical exposure and use and having had the same manufacturing and sterilization processes applied. By executing the simple and inexpensive tests listed in Table 5.2, material equivalency can be established. These tests provide a powerful way to evaluate and confirm equivalency with respect to mechanical, physical, chemical and toxicological properties for any proposed material. When performed regularly, these tests can serve as quality control measures and as test methods to evaluate and control change.

There are many benefits to performing these routine or quality control tests. When used regularly, they can minimize the risk of an adverse event or failure in the future. They can also provide the means to detect and evaluate known or suspected material changes, and they can be used to ensure that a material change will not compromise device quality or safety. Performing these tests routinely is prudent. It should lower costs during the life of the device and ensure compliance with FDA and ISO guidance documents. Finally, these test methods can reduce risk significantly and can provide assurance that the material and the medical device are safe for their intended use.

5.8 Risk assessment of extracts

One of the greatest challenges in medical device chemical characterization is performing adequate assessment of biological or toxicological risks from extractables that can compromise patient safety. ISO 10993-17 has clearly stated why and how risk assessments are a part of material biocompatibility

and are necessary for the assurance of biological safety.¹ Toxicological hazard is a property of the chemical constituents of the materials from which a medical device is made and should be considered in relation to the assurance of biological safety. Therefore, for a biological safety assessment, the first step involves chemical characterization of materials. Toxicological hazards can be identified from knowledge of the toxicity of materials or extracted chemicals.

A systematic analysis of biological risks is required using the general principles set out in clause 3 of ISO 10993-1. Unfortunately the matrix in ISO 10993-1 is often used as a checklist to perform a standard set of tests. What is actually needed is an appropriate scientific evaluation programme based on the specifics of the device.

The results of all tests should be interpreted in the context of the overall risk assessment to know whether a specific outcome indicates an acceptable risk or not. This collaborative approach emphasizes the need for an overall scientifically valid risk assessment. All parties involved, including the manufacturer, analytical chemists, and the toxicological risk assessor, must have input and be significant contributors to the assessment process.

Risk assessment of extracts or mixtures remains a complex problem. 'It is now recognized that significant data gaps exist in the area of mixtures toxicology and these can complicate accurate risk assessments.'¹⁵ If it is difficult to judge the risks associated with one pure substance, it becomes even more difficult if a solution or extract is a complex mixture of a number of different compounds. Most analytical chemists are acutely aware that leachable residue is likely to be a blend of different chemicals (Fig. 5.1). The resulting biological effect of combined exposure to several agents can be characterized as additive, supra-additive (synergistic) or infra-additive (antagonistic). Another type of interaction – potentiation (a special form of synergism) – may be observed. In cases of potentiation, one of two agents exerts no effect upon exposure, but when exposure to both together occurs, the effect of the active agent is increased. The assumption is made that compounds with similar metabolic pathways or even with similar structures will have an additive effective.

Sometimes, a small change in chemical structure produces sharply different toxicological effects. In addition, there is the possibility that mixtures will have a synergistic effect (i.e. far greater than additive, so that the risk to humans is magnified). Alternatively, the effect could be antagonistic, where the various residues cancel each other out.

5.8.1 Background information on risk assessments

Risk assessment is not new, but has only recently been publicized by international standards organizations and endorsed as an integral part of

chemical characterization and biocompatibility studies for medical devices. The suitability of a medical device for a particular use involves balancing any identified risks with the clinical benefit to the patient associated with its use. ISO 10993-17 states that 'among the risks to be considered are those arising from exposure to leachable substances arising from medical devices.' This standard provides a method for calculating maximum tolerable levels that may be used by 'other standards-developing organizations, government agencies, and regulatory bodies. Manufacturers and processors may use the allowable limits derived to optimize processes and aid in the choice of materials in order to protect patient health.' Risk assessment, as explained in ISO 10993-17, is really a decision-making tool that has evolved over time. Manufacturers and processors may use derived allowable limits to aid in choosing the most appropriate material for a particular medical device application. Toxicological risk assessments have a long history with strong ties to Europe (the BS 5736 series of standards), and the US FDA, Environmental Protection Agency and Occupational Safety and Health Administration. Now ISO 10993 standards for medical devices prescribe the use of toxicological risk assessments for biological studies including material characterization and degradation studies.

The risk assessment must be well organized, documented and evidence-based for effective use in support of decision-making with respect to product or material safety. The aim of the assessment should be to identify any biological hazards inherent in the materials used in the medical device and to estimate the risks resulting from these in light of the intended use. The goal is to develop a process that ultimately protects public health and establishes the safety of medical devices. This objective is supported by ISO 10993-17 in sub-clause 4.3 of the general principles for establishing allowable limits which states that 'the safety of medical devices requires an absence of unacceptable health risk.' The manufacturer of a medical device is responsible for assuring its biological safety, for documenting the assessment of toxicological risks, and establishing the effectiveness of the analysis. Evidence must be provided that an appropriate toxicological risk assessment has been carried out so that it can ensure that public health is not endangered. ISO 10993-17 also adds that 'where risk associated with exposure to particular leachable substances are unacceptable, this part of ISO 10993 can be used to qualify alternative materials or processes.' This is another example of the way risk assessment can be used as a mechanism for critical decision-making processes.

Additional information from biocompatibility tests or on the prior use of the materials may be used to provide a basis for further assessment of risks. Acceptable results from appropriate biological tests (e.g. those listed in the ISO 10993 series of standards) may give a degree of assurance that the risk of adverse reactions occurring during clinical use is low. These tests differ

from classical toxicity tests in that they typically attempt to mimic the conditions of clinical exposure to medical devices. Standardized toxicological tests are amenable to the generation and comparison of data from a wide range of test materials within or across chemical platforms. As standardized protocols must be broadly applicable for the study of a variety of different materials, they cannot realistically be expected at the same time to address highly focused mechanistic toxicological issues associated with only one or a few chemical compounds.¹⁶ This point of view was also expressed in UK Medicines and Healthcare products Regulatory Agency's updated Guidance Note 5 EC Medical Devices Directives: Guidance on the Biological Safety Assessment.¹⁷ The Guidance Note states that:

These tests, commonly termed biocompatibility tests, differ from basic toxicity tests in that they typically attempt to mimic the conditions of clinical exposure to medical devices and thus provide an indication of the probability of adverse effects arising during use. They tend, as a result, to be less sensitive than basic toxicity tests and are thus a less discriminating indicator of risk. Biocompatibility test data should therefore be used to complement an assessment based on materials characterisation, rather than as a replacement for it.

Toxicological hazard is a property of the chemical constituents of the materials from which a medical device is made and chemical composition should be considered in relation to hazard identification. Where significant risks arising from hazardous residues are identified by chemical characterization, their acceptance should be assessed in line with established toxicological principles. Biocompatibility tests identified in the ISO 10993 series of standards may be used to provide further assessment of risk.

5.8.2 Components of risk assessment

ISO 10993-17 is an ambitious, much needed guidance document that defines and documents consistent practices for evaluation of the risk factors for specific leachable substances. The probability that an adverse effect will arise from exposure to a chemical depends on its inherent toxicity, but also on the amount to which a subject is exposed and the route of that exposure. The standard provides a systematic method for assessing complex solutions or extracts. ISO 10993-17 uses four basic steps that are commonly used in the risk assessment process. These steps, defined by the National Academy of Sciences are:¹⁸

1. hazard identification,
2. dose-response assessment,
3. exposure assessment,
4. risk characterization.

These four steps, when accurately defined and evaluated, result in a statistically-derived probability that an adverse effect will occur at a defined exposure level. Risk characterization is the process in which the dose–response assessment and exposure assessments are integrated to predict risk to specific populations. Risk characterisation is the final stage in the risk assessment process and involves predicting the frequency and severity of effects in exposed populations.

In order to establish a tolerable intake for a specific leachable substance, modifying factors are applied to the data for non-cancer endpoints so that an appropriate intake value can be established. For example, the modifying factor is derived as the product of various component uncertainty factors. One example of a commonly used uncertainty factor is the factor used in extrapolating the effects of animal studies to humans. If only limited long-term exposure studies are available, a higher uncertainty factor leading to a lower acceptable exposure in the human population would be employed. It is noted in the standard that when this factor is combined with other uncertainty factors, modifying factors may be expected to differ by two orders of magnitude. Uncertainty factors and ultimately the modifying factors are derived on a case-by-case basis, and are highly dependent on the quality of the toxicological database.

An important step in any estimation of chemical toxicity is generating a dose–response curve, a graphic representation of the quantitative relationship between the level of exposure and the intensity or occurrence of a resulting adverse health effect. Figure 5.1 shows a typical dose–response relationship. A dose or concentration of a chemical substance that does not produce any adverse effect (i.e. No Observed Adverse Effect Level, NOAEL) is identified, usually from toxicological studies involving animals, but sometimes from epidemiological studies of human populations. A modifying factor is applied to the NOAEL to derive a Tolerable Daily Intake, the intake or concentration which is believed that a person can be exposed to daily over a lifetime without deleterious effect.

Manufacturing, assembling, packaging and sterilization of medical devices tend to result in a multiplicity of process chemicals that can potentially migrate into surrounding tissues and body fluids. Many of these are complex mixtures, often with poorly-defined toxicological profiles, will become increasingly important because moving from a chemical with well-established risks to a chemical where less is known can make it difficult to define the hazard, so a higher risk will be assigned.

The method outlined in ISO 10993-17, Method for the establishment of allowable limits for leachable substances, was used by the US FDA Center for Devices and Radiological Health (CDRH) to establish the tolerable intake for di(2-ethylhexyl)phthalate (DEHP) released from polyvinyl chloride-containing medical devices. ‘The safety assessment approach used

by the FDA/CDRH to derive the TI [tolerable intake] values is essentially identical to the method used by other regulatory agencies and advisory bodies to establish health protective exposure levels for DEHP (and other compounds).¹⁹ The process used to ascertain the safety of DEHP set the precedence for this approach, and is used to evaluate the safety or risk with regard to exposure to extracted chemicals. This process works well when dealing with a single chemical entity. However, as pointed out previously, antagonistic and synergistic effects are not accurately determined or predicted when multiple chemicals have been extracted. For this reason, biocompatibility tests listed in the ISO 10993 series of standards should be used to complement a risk assessment process as described in ISO 10993-17.

A second international standard, ISO 14971: 2007, Medical Devices – Application of risk management to medical devices, gives guidance with respect to evaluation of toxicological hazards.¹⁶ Annex I, Guidance on risk analysis procedure for biological hazards, also provides guidance with respect to toxicological hazards due to chemical constituents with the potential for causing biological harm. According to this international standard, in order to estimate toxicological risks, three major factors should be taken into account:

1. the chemical nature of the materials,
2. prior use of the materials,
3. biological safety data.

The amount of data required and the extent of the investigation is dependent upon the intended use/intended purpose and upon the nature and duration of patient contact. Therefore, material intended for the manufacturing of an implantable device would require a more extensive investigation than a surface device contacting intact skin.

Collectively, knowledge of the material's composition (including additives and processing aids), prior use of the material(s) in a predicate device or similar device, and biological safety tests should provide predictive evidence of any toxicological hazard to patients. While ISO 10993-17 can be used to establish allowable limits for individual chemicals, biological safety tests when used to complement the risk assessment can give another measure of assurance.

In practice it is not possible to carry out complete chemical characterization of a complex mixture obtained from extracts of device materials. Therefore, the integration of chemical and biological information is critical to any assessment of the toxicity of complex mixtures. While ISO 10993-17 deals with establishing allowable limits for each individual chemical, ISO 14971 relies on biological safety data as one of the factors to evaluate toxicological hazards. In combination, appropriate biological and chemical tests

provide a way to deal with some of the weaknesses of assessments of complex chemical substances.

Biological safety data provide another level of predictive evidence that none of the extracted chemical substances are potentially harmful to patients. *In vitro* tests, such as cytotoxicity and hemolysis, provide predictive evidence that extracted substances singularly and collectively are not toxic to mammalian cells. *In vitro* tests are very useful in studies of acute toxicity and also biotransformation products of extractables in that a large number of combinations of chemicals can be assayed using a single test article extract. The sample extract or mixture is treated as a whole and tested as is. Supporting data derived from *in vitro* and *in vivo* biological tests can help risk assessors make meaningful predictions as to a likely human response. Cell studies can help identify the mechanism by which a substance has produced an effect in the animal bioassay. These tests have the ability to predict any unexpected potentiation or synergistic effects not accounted for by ISO 10993-17 that may result in toxicity.

5.9 Conclusion and future trends

Almost all materials to be used in biomedical applications, and consequently nearly every one involved in the medical device industry, will require certain characterization tests. Material and chemical characterization forms the basis for understanding the composition of a medical device material and its potential to have an adverse biological effect when the device is put into use. It also serves as a means to ensure standardization of materials from one lot of devices to the next. As the harmonization of ISO 10993 standards and FDA requirements proceeds, the methods described above will be used by the device industry to a greater and greater extent to aid in the selection of optimal materials and to control the uniformity of medical products. This clearly establishes chemical and materials characterization as an essential part of medical device biocompatibility.

A comprehensive chemical characterization program that integrates the evaluation of extractables, device material stability and toxicological risk assessment provides predictive evidence of safety and effectiveness of the device and all its constituents. It is important to give consideration to any potential biological or chemical interactions between the biological environment and the device.

The integration of chemical and biological information is critical to the assessment of toxicity of device extracts. The guidance provided by ISO 10993-17 and ISO 14971 has made it clear that together, biological safety tests, knowledge of the material's composition (including additives and processing aids), along with prior use of the material(s) in a predicate device

or similar device should provide predictive evidence of any potential toxicological hazard to patients.

Once a risk assessment has been completed, the focus turns to risk management. Part 17 of ISO 10993 states that: 'Manufacturers and processors may use the allowable limits derived to optimize processes and aid in the choice of materials in order to protect patient health.' Decisions should be made utilizing the results of risk assessment, biological safety testing and safe clinical use of predicate devices as described in ISO 14971. When coupled or linked to biological safety testing, a successful biological safety assessment becomes a highly useful decision-making tool.

The implementation of chemical and material characterization requires close collaboration of chemists, material specialists, regulatory specialists and toxicologists. A team approach is imperative and will become even more important in the future as chemical and materials characterization continues to evolve and become even more important in the biological safety assessment of biomaterials and medical devices. With continued emphasis on chemical and material characterization, the role of chemistry as the central science connecting the physical sciences with life sciences and applied sciences will be even more important in the future development and evaluation of medical devices.

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Allowable limits for toxic leachables: practical use of ISO 10993-17 standard

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Abstract: This chapter describes an approach for setting tolerable intake (TI) values for chemical compounds released from medical device materials based on the method described in the ISO 10993-17 standard (*Biological evaluation of medical devices – Part 17: Establishment of allowable limits for leachable substances*). Practical advice is provided on how to derive both non-cancer and cancer-based TI values and recommendations are offered on how to account for potential toxicological interactions among the chemical constituents of an extract, how to derive a TI from an LD50 value (i.e., a dose associated with 50% death in exposed animals), and how to conduct route-to-route extrapolation of dose. The chapter also explores how the approach outlined in the ISO 10993-17 standard for derivation of TI values compares with methods developed by other groups for the establishment of safe exposure levels for environmental pollutants and impurities in drug substances and how to identify a key toxicity study to serve as the basis for the TI value.

Key words: tolerable intake, allowable limit, risk assessment, route-to-route extrapolation, mixtures, LD50, ISO 10993-17, leachables.

6.1 Introduction

The pre-clinical biological evaluation of medical devices typically involves testing of an extract of the device, components of the device, or the device itself using various biocompatibility tests. The ISO 10993-1 standard (*Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process*) provides a roadmap that outlines the biocompatibility testing recommended for various types of devices, depending on the duration of device contact with the patient and the type of tissues that the device comes into contact with. The latest version of the ISO 10993-1 standard describes an alternate approach for the biological evaluation of medical devices based

Note: The recommendations offered in this chapter should not be construed as guidance from the US Food and Drug Administration (FDA). The mention of commercial products, their sources or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

on identification and quantification of the leachable chemical compounds released from the device and an assessment of the potential toxicological risk posed by patient exposure to these compounds. Since this approach can often be conducted using toxicity data found in published literature, use of this method for the biological evaluation of some endpoints, notably systemic toxicity and carcinogenicity, may reduce the need for animal testing of the device, and may potentially shorten the time it takes for the device to get to market if long-term toxicity testing of the device in animals can be avoided. Despite the potential advantages, one of the practical limitations of implementing this biological evaluation method is the lack of toxicity data available for many of the compounds released from device materials. These toxicity data are needed to establish acceptable levels of patient exposure to each compound. The ISO 10993-17:2008 standard, *Biological evaluation of medical devices – Part 17: Establishment of allowable limits for leachable substances*, describes a method for deriving acceptable exposure levels (termed ‘tolerable intake’ or ‘TI’ values in the standard) for compounds released from medical devices; however, this method is best implemented when adequate toxicity data are available for the compounds of interest following toxicity testing by a clinically relevant route and duration of exposure. This is the case for well-studied compounds like di(2-ethylhexyl)phthalate (DEHP), a plasticizer released from PVC plastic, or ethylene oxide, a sterilant used for many medical devices. In contrast, repeat-dose toxicity studies by a clinically relevant route of exposure are not available for the overwhelming majority of compounds released from device materials. As a result, the need exists to provide practical guidance on how to derive TI values for compounds in the absence of ‘ideal’ toxicity data. It is equally important to provide guidance for when it is not feasible to implement this chemical characterization/risk assessment approach due to a lack of appropriate toxicity data and instead conduct the biological evaluation of the device using more traditional biocompatibility tests of the device or extracts. To address these needs, this chapter will attempt to provide general guidance on how to derive acceptable exposure limits using the procedure outlined in the ISO 10993-17 standard and will provide additional clarification on procedures that can be used to derive TI values in the absence of ideal toxicity data. This overview includes recommendations on how to:

- conduct route-to-route extrapolation of dose (a process necessary when toxicity data are not available from a clinically relevant route of exposure), but are available following administration of the compound by a different route of administration;
- account for potential toxicological interactions that may occur among complex mixtures of chemicals released from device materials; and
- derive acceptable exposure limit values when the only toxicity information available are LD50 values (doses associated with 50% death in exposed animals).

Since the ISO 10993-17:2008 standard already provides detailed guidance on how to derive TI values for compounds released from device materials, this chapter will not provide step-by-step guidance for this process. Also, the chapter does not provide detailed instructions on how to undertake some of the more complex risk assessment approaches (e.g., dose–response and pharmacokinetic modeling) mentioned in the standard, but rather points the reader to citations in the literature that provide more information on these approaches. Finally, the recommendations offered in this chapter should not be construed as guidance from the US FDA.

6.1.1 What is the ISO 10993-17 standard and how can it be used?

The ISO 10993-17 standard provides ‘a method by which maximum tolerable levels can be calculated from available data on health risks’. The first step in this process is derivation of a TI value for the compounds of interest. A TI value is defined as an ‘estimate of the average daily intake of a substance over a specified time period, on the basis of body mass, that is considered to be without appreciable harm to health’. The TI value, typically represented in units of mg/kg/day, can be represented as the allowable amount of a compound released from a device (mg/device) by taking into account the number of devices used to treat a patient per day that might release the compound, and the patient’s body weight. It is important to note the distinction between a TI value that is established for a specific chemical compound regardless of the type of device it is released from, and the Allowable Limit (AL) for the compound released from a specific device or class of devices. The latter takes into account non-science factors, such as device characteristics and the feasibility of achieving the TI, and as such is not strictly a health-based exposure limit (HBEL). As described more fully in the ISO 10993-17 standard, the TI serves as the basis for any device-specific AL for the compound; however, this chapter will not address the factors that go into determining the AL, but will instead focus on the risk assessment process of deriving the TI value from scientific data.

The ISO 10993-17 standard has been used as the framework for deriving TI values for compounds released from a wide range of device types. For example, as mentioned above, the method described in the standard was used to derive TI values for DEHP released from devices (US FDA, 2001) and also serves as the basis for the AL values for ethylene oxide and ethylene chlorohydrin in the ISO 10993-7:2008 standard, *Biological evaluation of medical devices – Part 7: Ethylene oxide sterilization residuals*. In addition, it can be used on a day-to-day basis to establish TI values used as part of a regulatory review of a new device, or even earlier in the device-development stage, to determine the most appropriate materials to use when manufacturing a device.

6.1.2 Similarities to other existing risk assessment guidance practices

The process described in the ISO 10993-17 standard for deriving TI values is similar or identical to the process used by regulatory agencies, advisory groups and standards bodies around the world to derive HBELs for chemical compounds. The basic approach for deriving TI values based on non-cancer endpoints; identification of an appropriate no-observed adverse effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) from a relevant toxicity study, and application of uncertainty factors to account for interindividual variability in response to the chemical compound in the human population (UF1), differences in potency between experimental animals and humans (UF2) and other data deficiencies (UF3); is identical to the approach that is well established and accepted in the risk assessment community (NAS, 2009). This approach forms the cornerstone of risk assessment approaches adopted by organizations such as the International Programme on Chemical Safety (IPCS, 2009), the International Conference on Harmonization (ICH, 2005) and the US Environmental Protection Agency (US EPA, 1993, 2005).

6.2 Process for setting tolerable intake (TI) values for compounds released from medical device materials

The ISO 10993-17 standard describes the general process for establishing TI values for compounds leached from device materials. As described below, key elements of this process include identifying a key study to serve as the basis for the TI value, calculation and application of a modifying factor to account for uncertainties in the data when the TI is based on non-cancer endpoints, and the use of various cancer risk assessment approaches to derive a cancer-based TI. Although the procedures to carry out these steps are outlined in the ISO 10993-17 standard, the sections below offer some practical guidance on how to implement these steps.

6.2.1 Selection of a key study to serve as the basis for the TI value

Derivation of a TI value for the compound of interest requires that the risk assessor carefully evaluate the available toxicological literature to identify the most appropriate study to base the TI on. The ISO 10993-17 standard is not very explicit in this regard, nevertheless the selection of a specific

toxicity study to serve as the basis for the TI should be justified and well documented in the risk assessment report. An example of a transparent and fully documented approach for selection of the key study upon which to base the TI can be found in the FDA safety assessment of DEHP (US FDA, 2001).

The characteristics of an appropriate toxicity study used as the basis for a TI value include:

- Administration of the compound by a clinically relevant route and duration of exposure,
- Use of multiple doses in the study to accurately characterize the dose–response relationship, ideally including a dose that produces an adverse effect and one that produces no effect (NOAEL),
- The use of appropriate control groups, adequate sample size, and statistical analysis of data.

Criteria for the selection of toxicity studies to serve as the basis for HBEL development have been established by various advisory panels, including the Annapolis Accords on the Use of Toxicology in Risk Assessment and Decision-Making (Gray *et al.*, 2001) and the National Toxicology Program (Shelby, 2005). Although these criteria are not explicitly described in the ISO 10993-17 standard, they may be useful for selecting key studies to serve as the basis for deriving TI values for compounds released from device materials.

Many toxicity studies have been conducted solely for hazard identification purposes, specifically, to determine if the compound produces an adverse effect at any dose. These types of studies are often conducted using only one dose and the data are generally insufficient for setting a TI value. Similarly, the results of *in vitro* toxicity studies should not be used as the basis for setting a TI value, but these data can be useful for elucidating the mechanism by which toxicity occurs or, in some cases, for defining an appropriate value for the uncertainty factors.

When many toxicity studies are available for a compound, it is often useful to prepare a table comparing the merits and limitations of each study being considered as the basis for the TI. An example of such a table is shown from the FDA safety assessment of DEHP (US FDA, 2001) (Table 6.1).

When multiple toxicity studies are available for the compound of interest, the TI value is typically derived from the study with the most sensitive endpoint (lowest NOAEL), assuming that the study is well conducted and the results are valid; however, TI values are not typically derived based on immunological endpoints and are not usually intended to be protective of immunological effects (e.g., hypersensitivity).

Table 6.1 Evaluation of critical toxicity studies: example of parenteral studies considered for derivation of TI for DEHP

| Study | Route | Effect(s) at LOAEL | Accept or reject study for TI derivation | Rationale |
|-------------------------------|-------|---|--|---|
| Jacobson <i>et al.</i> (1977) | IV | Histopathological changes in liver, altered BSP clearance kinetics | Reject | Questions about role of confounding factors (e.g., TB outbreak) |
| Fracasso <i>et al.</i> (1999) | IP | Peritonitis | Reject | Local effect |
| Nair <i>et al.</i> (1998) | IP | Reduced levels of Vitamin E in the liver | Reject | Not considered to be an adverse effect |
| Rubin and Chang (1978) | IV | Pulmonary effects | Reject | Questions about dose; only published in abstract form |
| Rutter (1973) | IV | Hepatomegaly, increased liver enzyme levels, increased lung weight | Reject | Questions about role of confounding factors (DEHP administered neat) |
| Curto and Thomas (1982) | IP | Reduced testicular Zn | Reject | Precursor event, not considered to be an adverse effect |
| Petersen <i>et al.</i> (1975) | IV | Reduced litter size following exposure of treated males to untreated females | Reject | Questions about dose |
| Greener <i>et al.</i> (1987) | IV | Reduced body weight gain, hepatomegaly, ↑SGOT | Reject | Questions about the way data were presented and statistical significance of differences |
| Cammack <i>et al.</i> (2003) | IV | Partial depletion of the germinal epithelium, decrease in diameter of seminiferous tubules; decreased testes weight | Accept | Appropriate for use |

SGOT, serum glutamic oxaloacetic transaminase.

Searching the literature to identify key studies

It is necessary to conduct a comprehensive search of the toxicological literature to identify the key study or studies to serve as the basis of the TI value. Too often, users of the standard will only conduct a cursory review of the literature on the toxicity of the compounds, an approach that may result in failure to identify important and relevant toxicity studies for the compounds of interest. At a minimum, bibliographic databases from the US National Library of Medicine (NLM), including the PubMed and TOXLINE databases, provide citations to papers in the literature and are very useful for identifying relevant toxicity studies. Factual databases in the NLM TOXNET family, such as ChemID Plus and the Hazardous Substances Databank (HSDB), provide compilations of toxicity values (e.g., LD50s) that may be useful as well; however, even if toxicity values are identified in factual databases, bibliographic databases should be searched as well to ensure that all relevant toxicity studies are identified.

The US EPA has recently released the ACToR database, which is described as 'EPA's online warehouse of all publically available chemical toxicity data.' Because of the comprehensive scope of the information it contains, the ACToR database may prove to be a useful resource for the toxicity data needed to derive or support a TI value.

Table 6.2 provides information on the data sources that may be useful for identifying key toxicity studies in the literature.

Often, Material Safety Data Sheets (MSDS) are used as a source of toxicity data for compounds released from device materials, and regulatory agencies may request an MSDS as part of a pre-clinical device submission dossier; however, the information provided in an MSDS is rarely sufficient to conduct an adequate toxicological assessment. As a result, even if an MSDS is identified for the compound of interest, additional efforts should be undertaken to identify other sources of toxicity information for the compounds of interest.

Table 6.2 Online resources for toxicity data

| Agency | Data source | How to access |
|---------------------------------|-------------------------------|---|
| US National Library of Medicine | PubMed and TOXNET | http://www.ncbi.nlm.nih.gov/pubmed / http://toxnet.nlm.nih.gov |
| University of California | Carcinogenic Potency Database | http://potency.berkeley.edu/ |
| US EPA | ACToR | http://actor.epa.gov/actor/faces/ACToRHome.jsp |
| US ATSDR | Toxicological Profiles | http://www.atsdr.cdc.gov/toxprofiles/index.asp |
| IPCS | CICAD and EHC documents | http://www.inchem.org/ |

Determining what constitutes an adverse toxicological effect

As described in the ISO 10993-17 standard, TI values are typically derived using information on the highest dose of the compound that does not produce toxicity in animal studies (the NOAEL) or in the absence of a NOAEL value, the lowest dose that produces adverse effects in the study (the LOAEL). The NOAEL is defined in the standard as ‘the greatest concentration or amount of a substance found by experiment or observation which causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure’. This definition acknowledges that relatively minor or adaptive biological effects, such as enzyme induction or changes in gene expression, can occur after exposure to a chemical compound, but these effects are not considered to be adverse and therefore should not serve as the basis for a NOAEL or LOAEL value. Since the NOAEL or LOAEL values are the doses associated with adverse effects, it is important to differentiate adverse from non-adverse effects that may be reported in a toxicity study. Various authors have commented on this issue. For example, Williams and Iatropoulos (2002) describe toxic or ‘adverse’ effects as those that can be lethal at the cellular or organism level, impair function or structure, diminish capacity to respond to stress, or may be irreversible. In contrast, less serious ‘adaptive’ effects include those that do not compromise viability at all levels of tissue organization, constitute potentially beneficial effects on function or structure, result in enhanced capacity to respond to stress and may include effects that are reversible. Other guidance on what constitutes an adverse toxicological effect has been provided in a series of papers by authors at the US Agency for Toxic Substances and Disease Registry (ATSDR) (Chou and Pohl, 2005; Pohl and Chou, 1985; Pohl *et al.*, 2005) and by other investigators (e.g., Lewis *et al.*, 2002). These additional sources of information may help the user of the standard determine which doses in a dose–response toxicity study should be selected as the NOAEL and LOAEL values.

Use of data from epidemiology studies

TI values are typically derived using toxicity data obtained from experimental animal studies; however, data from epidemiology studies may be available for the compound of interest. In fact, the standard notes that data from human studies are preferred over those from animal studies when setting a TI value. The results of epidemiology studies can serve as the basis for TI values, but the general limitations associated with the use of these studies for setting HBELs should be kept in mind, most notable being the lack of accurate exposure data associated with the adverse effect. In addition, a cause-and-effect relationship cannot be established from some types

of epidemiological studies. Therefore, as a practical matter, TI values are rarely derived using human data. Additional perspectives on the use of human data for setting HBELs has been offered by Nachman *et al.* (2011) and Raffaele *et al.* (2011).

6.3 Derivation of non-cancer TI values

The approach described in the ISO 10993-17 standard to derive TI values for non-cancer endpoints is essentially identical to that used by other regulatory agencies and advisory bodies to establish other types of HBEL values based on non-cancer endpoints. This method involves a two-step process: (1) identification of dose–response data from an appropriate toxicity study, as described above, and (2) application of uncertainty factors to account for interindividual variability in response to toxic compounds in the human population (UF1), differences in sensitivity to the effects of toxic compounds in experimental animals and humans (UF2), and deficiencies in the data (UF3) (Table 6.3). The process for selecting values for each of the UFs is described in more detail below.

6.3.1 Calculation of a modifying factor

The product of the factors used to account for uncertainty in the data is termed the ‘modifying factor’ (MF) in the standard. Typically, a default value

Table 6.3 Uncertainty factors for derivation of TI values

| Uncertainty factor designation | Range | Recommended default UF | Description |
|--|-------|------------------------|---|
| UF1, interindividual variability in the human population | 1–10 | 10 | To account for the variability in response between the mean of the healthy population and the response in some proportion of a sensitive subpopulation |
| UF2, interspecies extrapolation | 1–10 | 10 | To account for the possibility that humans are more sensitive to the adverse effects of a compound than experimental animals are |
| UF3, quality and relevance of the experimental data | 1–100 | None | To account for limitation in the toxicological data available for TI derivation, including absence of NOAEL value, absence of NOAEL from a long-term study, and lack of data from a clinically relevant route of exposure |

of 10 is used for uncertainty factors intended to account for differences in toxicological potency of the chemical between experimental animals and humans and variability in response of individuals in the population to the compound. The scientific basis behind the default values used for UF1 and UF2 are well established (Dourson and Stara, 1983; Renwick and Lazarus, 1998) and the process for selecting values for UF1 and UF2 is not as arbitrary as some may make it seem. Deviation from the default values of 10 for UF1 and UF2 should only occur when sufficient data are available to derive a scientifically valid alternate value. Sufficient data in this regard are pharmacokinetic and pharmacodynamic data that can be used to quantitatively estimate a human equivalent dose from animal data (UF1) or to characterize variability in response of the human population to the compound of interest (UF2). It should be noted that UF2 represents both pharmacokinetic and pharmacodynamic variability in the human population (Renwick and Lazarus, 1998). As a result, data provided on only one of these factors (e.g., enzyme polymorphism) is insufficient by itself to justify departure from the default value (e.g., Gundert-Remy *et al.*, 2002). Therefore, although the standard allows the user to select a value from 1 to 10 for UF1 and UF2, deviations from default value of 10 should occur only when substantial data exist to support an alternate value.

Concern has been expressed by some users of the standard, particularly in the regulated industry, that compounded conservatism exists in MF values, since upper-bound default values (10) are used for UF1 and UF2. This level of conservatism may be overstated, since it is important to keep in mind that the variability in response of the human population can exceed 10-fold, especially when comparing the response of critically ill patients to that of healthy individuals. Nevertheless, Gaylor and Kodell (2000) have described a 'Rule of 3' approach for accounting for compounded conservatism when multiple UFs are used to calculate a MF. This probabilistic approach provides scientific justification for an upper-bound MF of 300 when three uncertainty factors (e.g., UF1, UF2 and a factor to convert a LOAEL to a NOAEL) are needed to derive an HBEL.

The standard notes that the data used to derive exposure limits should be 'high quality and pertinent'; however, as noted above, toxicity data from well conducted and relevant studies are not available for many compounds released from device materials. Nevertheless, it is possible to derive TI values in the absence of 'ideal' critical toxicity studies, keeping in mind the uncertainty associated with these TI values. This includes the use of LD50 values as the basis for the TI and the use of data from studies in which the route of exposure was different from the clinically relevant route of exposure to the compound from the device. The standard provides some very general guidance on how to take these factors into account in the description of values for UF3, the uncertainty factor that deals with data quality.

The following additional considerations may be useful when deriving TI values from LD50 values and when data are not available from toxicity studies using a clinically relevant route of exposure.

Use of LD50 values as the basis for the TI

For many compounds released from device materials, data from repeat-dose toxicity studies are not available to serve as the basis for a TI. In the absence of these preferred data, acute lethality data (LD50 values) have been used as the basis for TI derivation. In such cases, the standard notes the following:

If only acute lethality data is available, a MF greater than 10 000 may be necessary to establish a TI for permanent contact. Any situation that results in a MF of greater than 10 000 is indicative of a high degree of imprecision in the analysis and consideration should be given, in such cases, to the urgent need for additional data.

Unlike the NOAEL, a value associated with no adverse toxicological effect, the LD50 is a dose associated with a very serious adverse effect, specifically, death to half of the exposed animals. Since the LD50 value represents a dose consistent with a very severe endpoint, it is understandable that a MF much greater than that applied to a NOAEL value should be used when deriving a TI based on an LD50 value rather than a NOAEL value.

Venman and Flaga (1985) compared oral LD50 values for compounds to their respective NOAEL values from long-term repeat-dose studies. The 95th percentile of the distribution of these LD50/NOAEL ratios was 0.0001, therefore, a NOAEL value can be conservatively estimated from an LD50 95% of the time using this conversion factor. Like any other toxicity study with a NOAEL, use of an additional MF of 100 is necessary to account for UF1 and UF2 when the NOAEL is estimated from an LD50 value. The product of the LD50-to-NOAEL conversion (1000) and the default values for UF1 (10) and UF2 (10) yields an overall conversion factor of 1×10^6 when estimating a TI from an LD50 value. Similarly Layton and colleagues (1987) recommended a factor of 5×10^6 to 1×10^5 to convert an oral LD50 to an equivalent Acceptable Daily Intake (ADI) value. Therefore, consistent with the guidance offered in the standard, it is necessary to use a factor greater than 10 000 when deriving a TI from acute lethality data, and there is considerable empirical support for the use of a conversion factor on the order of 1×10^6 when deriving a long-term TI value in this fashion; however, the conversion factor to derive a short-term TI based on an LD50 value may be less.

It is important to note that LD50 values should not be used to derive a TI value for compounds released from device materials when appropriate

data from repeat-dose studies are available. Also, any TI value derived using LD50 values should be considered to be interim or provisional until relevant data from repeat-dose toxicity studies are available to derive the TI.

Route-to-route extrapolation of dose

HBELs derived by various regulatory agencies and advisory groups are typically intended to protect individuals against adverse health effects that may occur following oral or inhalation exposure to a chemical compound. Although these routes may be relevant for some medical device-related exposures (e.g., oral exposure for dental devices), chemical constituents released from medical devices typically gain access to the systemic circulation via parenteral routes of exposure. Since the potency of compounds can differ markedly when administered via different routes of exposure, existing HBELs are not directly applicable for use in assessing the potential for compounds to produce adverse effects following parenteral exposure. The inability of risk assessors to use existing HBELs directly to assess the potential for a compound to produce adverse effects via a parenteral route of exposure suggests that parenteral HBELs should be derived from toxicity data obtained in studies employing a relevant route and duration of exposure. However, long-term parenteral toxicity data are unavailable for many compounds. In the absence of such data, it may be possible to derive provisional HBELs for parenteral exposure using existing data obtained following oral exposure of experimental animals or humans to the compounds of interest. To do so, it is necessary to estimate the parenteral dose of the compound that produces the same systemic effect as the oral dose. The most accurate method for conducting a route-to-route extrapolation of dose (short of conducting toxicity studies by both routes of exposure) is to develop a physiologically based pharmacokinetic (PBPK) model that explicitly takes into account the absorption, distribution, metabolism and elimination of the compound of interest and can estimate the target tissue dose of a compound following administration via various routes of exposure. Luu and Hutter (2000, 2001) have demonstrated the utility of this approach for medical device risk assessment. The standard encourages the use of PBPK models for conducting route-to-route extrapolation of dose; however, the necessary data and resources rarely exist to use PBPK models for this purpose. When available, bioavailability data can be used to estimate an equivalent dose between routes of exposure without the need for pharmacokinetic models; however, pharmacokinetic data are also typically not available to assess the bioavailability of many compounds released from device materials. Alternately, one of the most practical ways to conduct a route-to-route extrapolation of dose is to assess functional bioavailability by comparing LD50 values from different routes of exposure.

For example, an equivalent NOAEL value from a clinically relevant route of exposure, say intraperitoneal (IP), can be estimated from a NOAEL from a toxicity study that used a non-clinically relevant route of exposure (e.g., oral), and the ratio of the LD50 values for each of the routes of exposure:

$$\text{NOAEL}_{\text{IP}} = \frac{\text{LD50}_{\text{IP}}}{\text{LD50}_{\text{ORAL}}} \times \text{NOAEL}_{\text{ORAL}} \quad [6.1]$$

The lowest reported LD50 values from each route of exposure should be used for this dose conversion and the values must be obtained from studies that used the same experimental animal species. Use of this approach requires the assumption that the bioavailability of the compound after short-term, high-dose exposure, typical of LD50 studies, is equivalent to that for longer-term, lower-dose studies, more typical of the repeat-dose toxicity studies used to derive NOAEL values.

In the absence of data that would permit route-to-route extrapolation of dose on the basis of pharmacokinetic data or the estimation of functional bioavailability based on LD50 values, default conversion factors have been used to estimate the equivalent dose of a compound by different routes of exposure. For example, the USP (US Pharmacopeial Convention) (2010) used a default conversion factor of 0.1 (i.e., default assumption of 10% oral bioavailability) to estimate permissible daily exposure (PDE) values for metal impurities in parenteral drugs from equivalent oral exposure values.

Route-to-route extrapolation techniques are intended to estimate equivalent *systemic* doses across routes of exposure. The dose associated with portal-of-entry effects or *local* effects seen at the site where the toxic compound first contacts the body (i.e., nasal mucosa in an inhalation study) does not typically serve as the basis for route-to-route extrapolations of dose, with some exceptions (e.g., Morris *et al.*, 1996). Therefore, route-to-route extrapolation is generally not performed to estimate equivalent doses that produce adverse effects following local or direct contact exposure to compounds released from devices (i.e., estimation of a tolerable contact limit (TCL) from one site (e.g., mucosa) from data from another (e.g., skin)).

Use of toxicity data from structural analogs

In the absence of toxicity data on the compound of interest, some risk assessments have been conducted using data from compounds that are structurally related to the compound of interest, based on the assumption that the toxicity of the structural analog will be similar to that of the subject compound. In many cases, structural analogs can produce a toxic effect similar to the compound of interest, at least qualitatively, but justification should be

provided that the potency of a structural analog is equivalent to or greater than that of the subject chemical. The selection of a structural analog with toxicity data to serve as a surrogate for a compound without data should not be made arbitrarily; rather, the process for identifying structurally and toxicologically similar chemicals should be justified. The approach outlined by the OECD (2007) and others (e.g., Wu *et al.*, 2010) may be useful for justifying the selection of toxicologically similar compounds for the risk assessment.

6.3.2 Dose–response modeling as an alternative to the NOAEL/UF approach for non-cancer risk assessment

Benchmark dose (BMD) modeling has been developed as an alternate to the traditional NOAEL/UF factor approach for deriving non-cancer HBEL values (US EPA, 2000). Like the NOAEL/UF approach, UFs are applied to a ‘point of departure’ on the dose–response curve to derive the HBEL; however, the BMD approach uses computational models to identify a specific point on the dose–response curve (e.g., lower limit on the 5% response) to serve as the basis for the derivation of the exposure limit. In contrast, a point of departure such as a NOAEL or LOAEL value from a study is determined by the doses selected by the investigators conducting the study. Although the BMD method was first proposed in the mid-1980s (e.g., Crump, 1984), and was widely used by the US EPA when the ISO 10993-17 standard was written (US EPA, 2000), it has only recently gained more widespread international acceptance as a means to derive non-cancer HBEL values (e.g., EFSA, 2009; Filipsson *et al.*, 2003), and revisions of the standard will likely include the BMD approach as an alternative to the NOAEL/UF approach for deriving non-cancer TI values. The recent review by Davis *et al.* (2011) provides a useful overview of the BMD approach and describes differences in its use between the USA and Europe.

6.4 Derivation of cancer-based TI values

The standard describes a weight-of-evidence approach for determining which risk assessment approach is appropriate for setting a cancer-based TI for a compound depending on whether the compound exerts its carcinogenic effect via a genotoxic (non-threshold) or non-genotoxic (threshold) mechanism of action. The TI for non-genotoxic carcinogens can be derived using the same NOAEL/MF approach used for non-carcinogens; however, the standard offers flexibility in the approach used for the cancer risk assessment of genotoxic carcinogens, depending on which risk management approach is preferred.

Approaches used to assess the potential cancer risk posed by exposure of individuals to compounds released from FDA-regulated products are outlined by Gaylor *et al.* (1997). Typically, a simple, linear extrapolation method is used to estimate excess cancer risk from dose–response data. The use of a simple linear extrapolation technique provides a practical means to estimate low-dose cancer risks without the need for computational models. In this approach, the dose associated with a given excess cancer risk, say 10^{-5} or 1 in 100 000, can be estimated from a point on the dose–response curve in a carcinogenicity study. For example, if administration of a compound at a dose of 20 mg/kg/day produced tumors in 15 out of 50 animals (30% tumor incidence or 0.3) following lifetime exposure and the control group had 5 tumors in 50 animals (10% tumor incidence or 0.1), the excess tumor incidence at this dose would be 20% (0.2). Assuming a linear non-threshold response at lower doses, the dose of the compound associated with a 10^{-5} (0.00001) excess tumor incidence would be:

$$\frac{20 \text{ mg/kg/day}}{0.2} = \frac{x \text{ mg/kg/day}}{0.00001} \quad [6.2]$$

$$x = 0.001 \text{ mg/kg/day}$$

Any dose associated with a low-dose tumor incidence in experimental animals (e.g., 10^{-5}) should then be extrapolated to a human equivalent dose using appropriate extrapolation methods (e.g., Mahmood, 2007; Travis, 1991).

Other statistical approaches to perform high-to-low dose extrapolation are available for cancer risk assessment, as described in the US EPA's Cancer Risk Assessment Guidelines (EPA, 2005); however, use of an approach other than those described in the standard should be justified.

When deriving a cancer-based TI, the ISO 10993-17 standard directs the user to estimate the dose of the compound associated with a 10^{-4} or 1 in 10 000 excess cancer risk. It was the intent of the working group writing the standard at the time to use this specific excess cancer risk value as a starting point for the analysis. Regulatory agencies and advisory groups have used excess cancer risk values ranging from 10^{-3} to 10^{-6} as the basis for risk management decisions (e.g., Rhomberg, 1996). Factors such as the size of the affected patient population and the nature of the device from which the compound is released can be taken into account when setting a cancer-based TI value.

6.4.1 Method to derive a cancer-based TI from TD50 data

Gaylor and Gold (1995) have proposed a method to estimate a virtually safe dose (VSD) for carcinogens based on the relationship between the slope of

the dose–response relationship in a two-year bioassay of the compound and the dose that produces tumors in 50% of animals (TD50) reported in the carcinogenic potency database (CPDB, 2012). The VSD is equivalent to the dose of the compound associated with 10^{-6} excess cancer risk and can be estimated as follows according to Gaylor and Gold (1995):

$$\text{VSD} = 10^{-6} \times \frac{\text{TD50}}{0.87} \quad [6.3]$$

Although this approach is not described in the ISO 10993-17:2008 standard, it does represent a practical way to derive a cancer-based TI for compounds that have TD50 values in the CPDB without the need to perform dose–response modeling.

6.4.2 Other considerations for cancer risk assessment

Compounds released from implanted devices may exert carcinogenic effects at the site of implantation as well as distant to the implant. It is important to keep in mind that the chemical characterization/risk assessment approach can only be used to assess the potential for systemic carcinogenic effects to occur, not local tumor formation at an implant site, since local tumor formation may be influenced by additional factors such as the physical form of the material.

To accommodate the needs of regulatory agencies in countries that do not typically accept linear extrapolation as a default approach for the risk assessment of genotoxic carcinogens, the standard provides the user with the option of reducing patient exposure to the potentially carcinogenic compound to a level as low as reasonably practicable. Under this approach, it is not necessary to derive a cancer-based TI for the compound of interest; rather, risk is managed using other approaches, such as through appropriate labeling of the device or other risk communication tools.

A threshold of toxicological concern (TTC) approach has been used for many years as a practical tool to identify acceptable levels of exposure to chemical compounds. The TTC is defined by Kroes *et al.* (2004) as the, ‘human exposure threshold value for all chemicals, below which there would be no appreciable risk to human health’. Since the TTC value is intended to be protective for all compounds (with the exception of very potent carcinogens), any value selected must be conservative (low) to be appropriately protective. The TTC concept is well established in various regulatory agencies and serves as the basis for the US FDA’s Threshold of Regulation for indirect food additives (US FDA, 1995) and the limits on genotoxic impurities in drug products set by the EMEA (2006). Application of the TTC approach for compounds released from device

materials is currently under consideration by the ISO TC/194 working group on allowable limits for leachable compounds. Until additional guidance is available on the use of the TTC approach for compounds released from device materials, the user may find the information on application of the TTC concept to impurities in food additives (Felter *et al.*, 2009), drug products (Ball *et al.*, 2007) and personal care products (Blackburn *et al.*, 2005) to be useful. Once implemented, it may be possible to use a TTC value for the cancer risk assessment of compounds released from device materials if dose–response data are not available to derive a compound-specific TI.

As noted above, the standard recommends the use of PBPK models to estimate the delivered dose of the compound to the target tissue whenever possible. The use of these models for the biological evaluation of devices is addressed in the ISO 10993-16, *Biological evaluation of medical devices – Part 16: Toxicokinetic study design for degradation products and leachables*, and from a device risk assessment perspective, these models are perhaps most useful for estimating equivalent dose across various routes of exposure (e.g., Fisher *et al.*, 2011; Luu and Hutter, 2001). However, as a practical matter, development of these models requires specific expertise and the models themselves require a great deal of pharmacokinetic data. Therefore, despite the recommendation in the ISO 10993-17 standard, it is not often practical to use PBPK models to define delivered dose for the cancer risk assessment of compounds released from device materials.

When conducting a cancer risk assessment, attention should be given to the form of the compound being tested in a carcinogenicity study and the form of the compound released from the device. For example, the variability in the carcinogenicity of different forms of nickel is thought to be due to differences in bioavailability of the metal to the nucleus (Goodman *et al.*, 2011). Similarly, hexavalent chromium has been shown to be carcinogenic, whereas trivalent chromium is not. The valence of chromium is particularly important to consider when evaluating the carcinogenic potential of chromium ions released from devices made from chromium-containing alloys (Keegan *et al.*, 2008).

6.5 Derivation of TI values for local effects

The ISO 10993-17 standard describes the derivation of a TCL, a value intended to be protective for chemically induced local irritation. The TCL value is conceptually similar to the TI, since it requires dose–response data to identify a non-irritating (NIL) or minimally irritating (MIL) amount of the compound, and it involves application of uncertainty factors to calculate the tolerable level of exposure; however, unlike the TI, the units are expressed on a surface area basis (e.g., mg/cm²).

When deriving a TCL, attention should be paid to using data obtained from studies conducted using exposure conditions that are clinically relevant to the device under review. For example, the potential for a compound released from a wound dressing to produce adverse effects is probably best assessed in an irritation study where the exposure site was occluded.

As a practical matter, the potential for chemical compounds released from devices to produce irritation is typically assessed by testing the device or extract of the device using an approach similar to that described in the ISO 10993-10:2010 standard (*Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization*) or similar approach. Adequate dose–response data for irritation are rarely available to establish a TCL for compounds released from device materials. It should be noted that the TCL is not protective for irritant effects that occur by mechanical means or an interactive effect between chemical and mechanical irritation.

6.6 Other issues to consider

6.6.1 Use of HBEL values derived by other regulatory agencies and advisory groups

Many of the compounds released from device materials (e.g., solvents, plasticizers) are also found as contaminants in pharmaceuticals, released from food packaging materials, or are found as environmental contaminants. In addition, exposure to these compounds can occur in occupational settings. As a result, various regulatory agencies and advisory groups have established HBELs for compounds that may be released from devices. HBEL values derived by other regulatory agencies and advisory groups include minimum risk levels (MRLs) derived for pollutants at hazardous waste sites by the US ATSDR, reference dose (RfD) and reference concentration (RfC) values for environmental pollutants derived by the US EPA, and PDE values for impurities in drug substances derived by the ICH. Additional HBEL values derived by regulatory agencies are provided in Table 6.4.

Before deriving a TI value *de novo* for compounds released from device materials, it is useful to determine if a HBEL for the compound has already been established. It is important to keep in mind that HBEL values derived by regulatory agencies and advisory bodies are typically calculated using toxicity data from oral or inhalation routes of exposure. As described above, if an oral or inhalation HBEL value is available, it may be necessary to derive an equivalent dose for the clinically relevant route of exposure by using appropriate route-to-route extrapolation techniques. Nevertheless, even if an existing oral or inhalation HBEL is available and an equivalent parenteral TI can be established, it is important also to search the literature to determine if toxicity data from the clinically relevant route of exposure

Table 6.4 HBEL values derived by regulatory agencies and advisory groups

| Agency/advisory group | Value | How to access |
|-----------------------|--|---|
| California OEHHA | No Significant Risk Level (NSRL) and Maximum Allowable Dose Level (MADL) | http://oehha.ca.gov/prop65/getNSRLs.html |
| Health Canada | Tolerable Intake (TI) and Tolerable Concentration (TC) | http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/value-valeur/index-eng.php |
| ICH | Permissible Daily Exposure (PDE) | http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html |
| RIVM | Tolerable Daily Intake (TDI) | http://www.rivm.nl/bibliotheek/rapporten/711701025.pdf |
| US ATSDR | Minimum Risk Level (MRL) | http://www.atsdr.cdc.gov/mrls/index.asp |
| US EPA | Reference Dose (RfD) and Reference Concentration (RfC) | http://www.epa.gov/IRIS/ |

are available for derivation of the parenteral TI. In addition, caution should be exercised when using occupational exposure values as the basis for TI derivation, as many of these limits are based on factors other than health protectiveness (e.g., technical feasibility of reaching the limit, odor threshold, etc.) or are based on protection against portal-of-entry effects (e.g., local respiratory effects) that may not be relevant for the route by which the patient is exposed to the compound from the device.

6.6.2 Mixtures

TI values are typically established for individual compounds; however, patients can be exposed to a complex mixture of compounds released from device materials. A number of risk assessment approaches have been proposed to account for potential interactive toxicological effects (e.g., Teuschler, 2007) and one of these approaches, the Hazard Index approach, is described in Annex B of the standard. The user of the standard is encouraged to consider the potential for interactive toxicological effects to occur among the compounds leached or extracted from a device when deriving TI values for each individual compound. The potential for synergistic effects to occur is an important consideration from a public health protection standpoint; however, it is important to note that interactive toxicological effects

are much less likely to occur when exposure occurs at doses well below the NOAEL of each component of the mixture (e.g., Boobis *et al.*, 2011; Feron *et al.*, 1998; Seed *et al.*, 1995). Such low-dose exposure conditions are often present when leachables are released from medical device materials.

6.6.3 Need for transparency and justification of values in the risk assessment

Like some of the other biocompatibility standards in the ISO 10993 series, the 10993-17 standard does not describe a proscriptive approach for the biological evaluation of medical device materials. There are no pass/fail criteria in the standard and the user is expected to exercise sound scientific judgment in the application of these principles. As a result, two users of the standard could derive very different TI values for the same compound using information from the same data set. Along these lines, this chapter does not advocate a specific risk assessment approach, but simply points out the merits and limitations of approaches described in the standard, as well as alternate approaches that may be useful. Because of the various approaches that can be used to derive a TI value, justification should be provided in the risk assessment report on the selection of the specific approaches used to derive the TI value. Sufficient detail should be provided to give a clear explanation for the critical study or studies selected to serve as the basis of the TI, for the values selected for uncertainty factors, especially if there is a departure from the suggested default values, and for cancer risk assessment, the specific approaches used for high-to-low dose and interspecies extrapolation of dose. The need to carefully describe and document the risk assessment approach used is especially important when it is necessary to extrapolate equivalent doses across durations or routes of exposure, when the TI is based on an LD50 value, or when toxicity data from a surrogate compound is used.

6.7 Conclusion

With the growing acceptance of the chemical characterization/risk assessment approach for the biological evaluation of medical devices, there is an increasing need to derive TI values for compounds released from device materials. These TI values are derived from toxicity data typically obtained from studies conducted in experimental animals. Although adequate toxicity data are available to set TI values for some well-studied compounds, data are not typically available for many of the compounds released from devices. As a result, additional guidance beyond that offered in the ISO 10993-17 standard is useful for deriving TI values when only limited toxicity

data are available. It is important to note that considerable uncertainties are associated with the use of less than ideal data sets for the derivation of TI values. Consequently, the user of the standard should consider conducting the biological evaluation of the device in other ways (e.g., biocompatibility testing of an extract) if insufficient toxicity data exist to implement the chemical characterization/risk assessment approach. Similarly, any TI values derived using less than ideal toxicity data (different duration or route of exposure, LD50 values), should be considered to be provisional or interim until more relevant data can be obtained to derive the TI.

Despite these limitations, the chemical characterization/risk assessment approach has been used successfully for the biological evaluation of many medical devices, and the process for establishing the TI values necessary for this approach is well documented in the ISO 10993-17 standard. Although the use of this approach for the biological evaluation of medical devices is in its early stages, the general method described in the standard for deriving TI values is well accepted and essentially the same as that used by regulatory agencies and advisory groups for establishing safe levels of exposure to compounds in environmental, occupational and consumer settings.

The science and practice of risk assessment are undergoing exciting changes intended to incorporate scientific information into the decision-making process more efficiently through the Tox21 program and other initiatives (e.g., Krewski *et al.*, 2010; Rhomberg, 2010). New methods to assess the risk posed by exposure to chemical compounds are rapidly evolving and many of these new and promising approaches have been reviewed by the US National Academy of Sciences (Abt *et al.*, 2010; NAS, 2009) and other advisory bodies. As the ISO 10993-17 standard undergoes revision, these new risk assessment approaches will be considered for incorporation into the standard.

6.8 References

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In vivo and *in vitro* testing for the biological safety evaluation of biomaterials and medical devices

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Abstract: Medical devices and the materials they are composed of need to be evaluated for their safety within the context of a risk management process. Safety issues related to toxicity can be evaluated using the ISO 10993 series of international standards for biological evaluation of medical devices. It provides an approach that combines the review and evaluation of existing data from all sources with, where necessary, the selection and application of additional tests, thus enabling a full evaluation to be made of the biological responses to each medical device, relevant to its safety in use. This chapter focuses on the various test methods, both *in vitro* and *in vivo*, that can be used for this evaluation. In addition to general *in vitro* cytotoxicity tests, more specific *in vitro* tests for genotoxicity, interaction with blood, and irritation are highlighted. Furthermore, *in vivo* tests for irritation and sensitization, haemocompatibility, genotoxicity, implantation and systemic toxicity are described in detail.

Key words: medical devices, biomaterials, ISO 10993 series, biological evaluation, *in vitro* testing, *in vivo* testing.

7.1 Introduction

Like all products intended to be used in humans, medical devices, and the materials they are composed of, need to be evaluated for their performance and their safety. Safety evaluation should be performed within the context of a risk management process, such as described in the international standard ISO 14971 for the application of risk management to medical devices (ISO 14971: 2007). To minimize the risks involved in the use of the device, all known or foreseeable hazards should be identified, and the risks arising from the identified hazards should be estimated and evaluated. The risks should be controlled by eliminating or reducing them as far as possible, aiming for inherent safety by design. Further reduction of risks to an acceptable level should be pursued by using protective measures in the medical device itself

or in the manufacturing process. This is an iterative process, which should be repeated when indicated, for example, due to changes in product specifications such as a change in source material or reported adverse effects of its components (Fig. 7.1).¹ If the evaluation of the benefit of the device versus the residual risks is favourable, these risks should be addressed on the label and in the instructions for use (IFU) as warnings, precautions or contraindications.

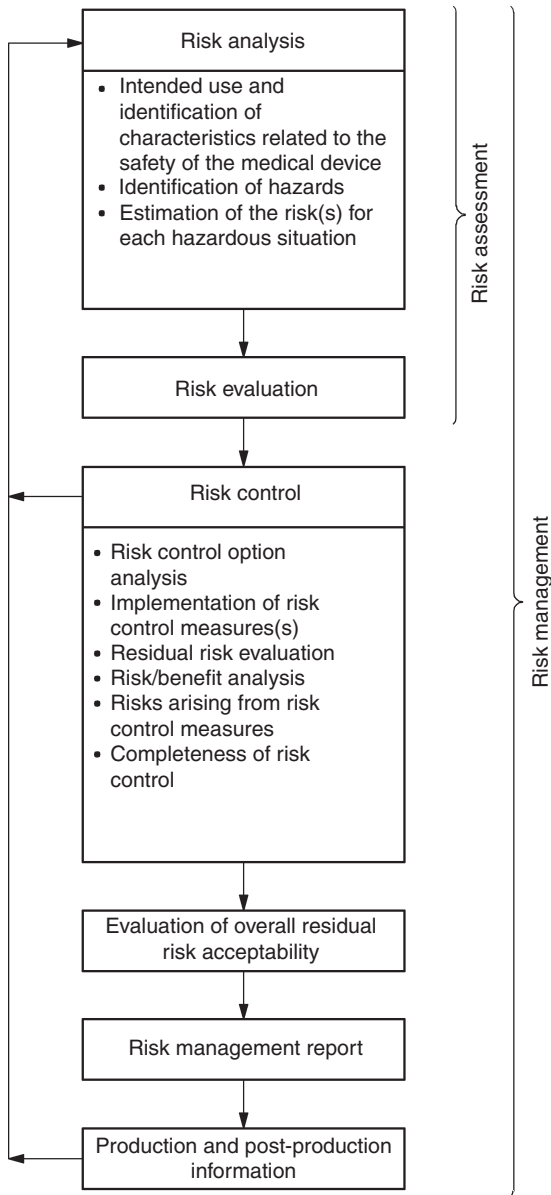
An important part of the identified hazards and associated risks will be related to biological safety aspects, both for toxicity issues and for infectious disease agents. This chapter is specifically addressing the testing of biomaterials and medical devices in the context of a biological safety evaluation with regard to toxicity issues as input for part of the risk analysis indicated as 'identification of hazards' and 'estimation of the risk(s) for each hazardous situation' in Fig. 7.1. The ISO 10993 series of international standards provides a framework for this. Part 1 of the series is intended to be a guidance document. It provides an approach that combines the review and evaluation of existing data from all sources with, where necessary, the selection and application of additional tests, thus enabling a full evaluation to be made of the biological responses to each medical device, relevant to its safety in use. Other parts of the ISO 10993 series include standards and technical reports that describe the various additional tests that may need to be performed for the full biological safety evaluation of biomaterials.

7.2 Pre-testing considerations

The evaluation of the biological safety of a medical device should be a strategy planned on a case-by-case basis to identify the hazards and to better estimate the risks of known hazards. The testing strategy should include a rationale for the selection and/or the waiving of tests. The rationale should be a clear, concise, logical and scientifically reasoned plan for evaluating biological safety that demonstrates that all hazards have been considered and relevant risks evaluated and controlled (ISO 10993-1:2009). A summary of this process is shown in Fig. 7.2.

Before starting any biological safety evaluation, the identity of the material to be tested has to be known. Thus, a proper characterization of the biomaterial is very important (see Chapter 5). The extent of chemical characterization required depends on what pre-clinical and clinical safety and toxicological data exist, and on the nature and duration of body contact with the medical device. However, as a minimum, the characterization

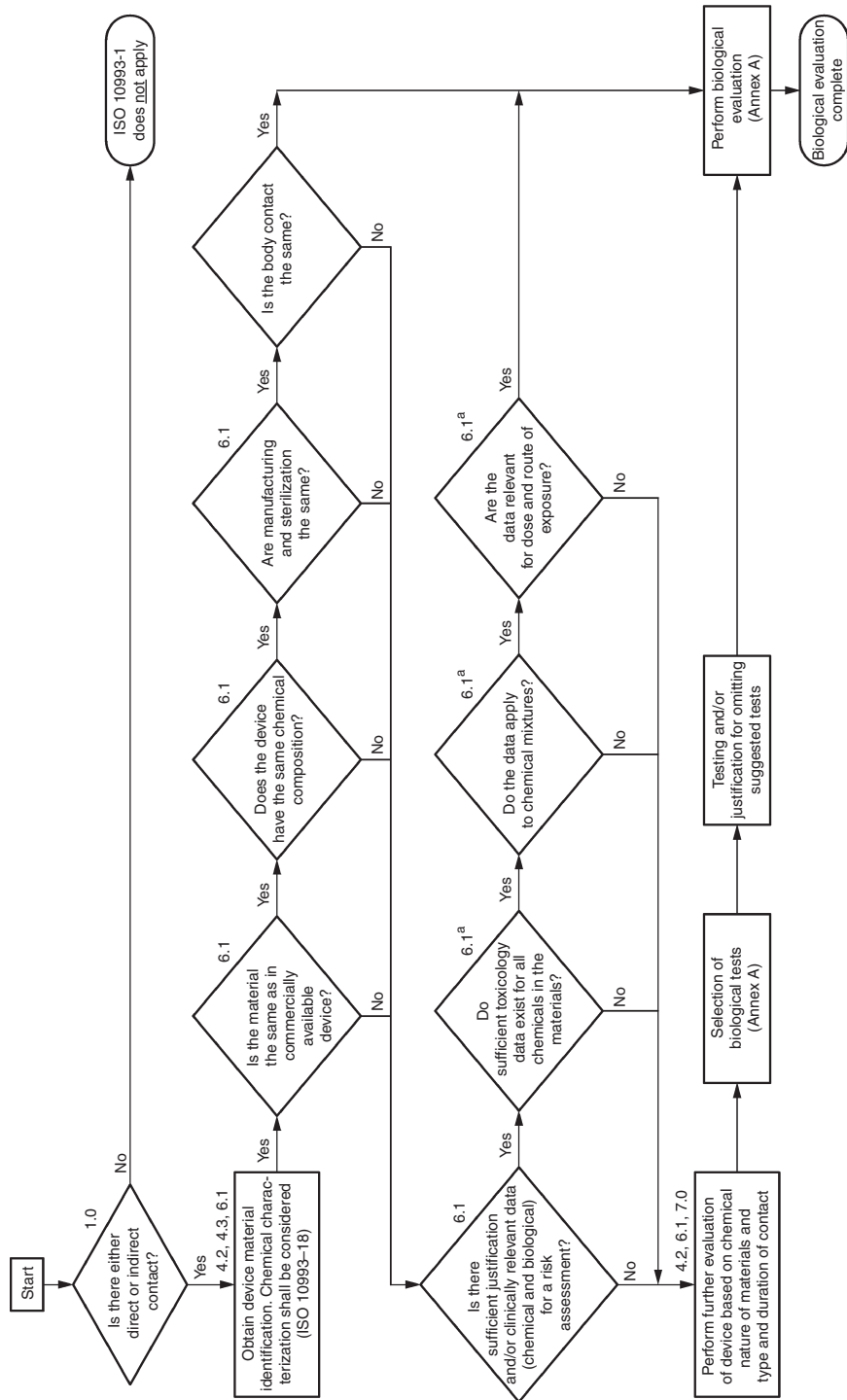
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7.1 A schematic representation of the risk management process (ISO 14971:2007)

should address the constituent chemicals of the device and possible residual process aids or additives used in its manufacture.

The information on the chemical composition of a material may be used to identify potential hazards associated with certain components of the material



7.2 Summary of the systematic approach to a biological evaluation of medical devices as part of a risk management process (ISO 10993-1:2009). The numbers in the figure refer to the clauses as indicated in ISO 10993-1:2009.

already in an early stage of development, before any testing has been done. Part of the material characterization should also be an assessment of the potential for exposure to the various identified components. Relevant guidance for the entire characterization, including the potential for degradation of materials used in medical devices, such as polymers, metals and ceramics, is described in parts 9, 13, 14, 15, 18 and 19 of the ISO 10993 series. Information on potentially toxic components can be gathered from literature or from additional testing. Based on this, a risk assessment can be performed with regard to allowable limits of that component in the medical devices (see Chapter 6 and ISO 10993-17). For devices that have known leachable chemical mixtures, potential synergies of the leachable chemicals should be considered.

In addition to this classical safety evaluation for chemical constituents, a safety evaluation of the final products and/or solid materials relevant to their intended use needs to be performed. All reasonably and practicably available information should be gathered and compared to the data set(s) needed to assess the biological safety of the device. When sufficient information is available in the literature, additional *in vivo* testing should be avoided, thus reducing the use of animal testing in accordance with the ISO 10993-2 standard on animal welfare. When the existing pre-clinical and clinical data, including history of safe use, meet the requirements of biological evaluation, further animal testing would be unethical. In assessing the relevance to the biological evaluation of data on prior use of a material the level of confidence in the historical data should be taken into account. ISO 10993-18:2005, Annex C, gives some informative principles for judging toxicological equivalence (ISO 10993-18:2005).

For the identification of any additional testing, ISO 10993-1 gives guidance on the possible assays that may need to be performed to demonstrate safety of a medical device or biomaterial. Table 7.1 (ISO 10993-1:2009 Annex A) indicates assays to be considered, based on the use of a medical device (on the surface, as external communicating device, or as implant), the contact site (mucosal surfaces, blood or tissues), and the contact time (limited (≤ 24 h), prolonged (> 24 h but ≤ 30 days) or permanent (> 30 days)). It should be realized that depending on the type of medical device and its application, a range of assays can be selected. The information presented in ISO 10993-1 should not be considered as a checklist for assays that are mandatory to be performed in order to demonstrate safety. Due to the diversity of medical devices, not all tests identified in a category will be necessary for a particular device. On the other hand, additional tests not indicated in the Table 7.1 may be necessary in order to complete the risk assessment. For most devices at least the following tests have to be considered: cytotoxicity, sensitization and irritation/intracutaneous reactivity. Especially when contact with blood or internal organs can be expected, systemic acute and subchronic toxicity testing, genotoxicity, implantation and haemocompatibility testing also may

Table 7.1 ISO 10993-1:2009 (Annex A) indicates assays to be considered, based on the use of a medical device (on the surface, as external communicating device, or as implant), the contact site (mucosal surfaces, blood, or tissues), and the contact time (limited (≤ 24 h), prolonged (> 24 h but ≤ 30 days), and permanent (> 30 days))

| Medical device categorization by | | | Biological effect | | | | | | | |
|----------------------------------|---------------------------------|--|-------------------|---------------|---|---------------------------|---|--------------|--------------|--------------------|
| Nature of body contact (see 5.2) | | Contact duration (see 5.3) A – limited (≤ 24 h) B – prolonged (> 24 h to 30 days) C – permanent (> 30 days) | Cytotoxicity | Sensitization | Irritation or intracutaneous reactivity | Systemic toxicity (acute) | Subchronic toxicity (subacute toxicity) | Genotoxicity | Implantation | Haemocompatibility |
| Category | Contact | | | | | | | | | |
| Surface device | Skin | A | X ^a | X | X | | | | | |
| | | B | X | X | X | | | | | |
| | | C | X | X | X | | | | | |
| | Mucosal membrane | A | X | X | X | | | | | |
| | | B | X | X | X | | | | | |
| | | C | X | X | X | | X | X | | |
| | Breached or compromised surface | A | X | X | X | | | | | |
| | | B | X | X | X | | | | | |
| | | C | X | X | X | | X | X | | |
| External communicating device | Blood path, indirect | A | X | X | X | X | | | | X |
| | | B | X | X | X | X | | | | X |
| | | C | X | X | | X | X | X | | X |
| | Tissue/bone/dentin | A | X | X | X | | | | | |
| | | B | X | X | X | X | X | X | X | |
| | | C | X | X | X | X | X | X | X | |
| | Circulating blood | A | X | X | X | X | | | | X |
| | | B | X | X | X | X | X | X | X | X |
| | | C | X | X | X | X | X | X | X | X |
| Implant device | Tissue/bone | A | X | X | X | | | | | |
| | | B | X | X | X | X | X | X | X | |
| | | C | X | X | X | X | X | X | X | |
| | Blood | A | X | X | X | X | X | | X | X |
| | | B | X | X | X | X | X | X | X | X |
| | | C | X | X | X | X | X | X | X | X |

^aThe crosses indicate data endpoints that can be necessary for a biological safety evaluation, based on a risk analysis. Where existing data are adequate, additional testing is not required.

be needed depending on the time of the contact with the patient. Additional tests not indicated in the table may include biodegradation and toxicokinetic studies, chronic toxicity, carcinogenicity, immunotoxicity, neurotoxicity or reproductive/developmental toxicity.

7.3 Sample preparation

Compared to classical safety evaluation for chemicals, the safety evaluation of solid products and/or materials as they are used in medical devices poses a specific challenge to the toxicologist. It can be imagined that the use and administration of any material to be tested in the various biological assays available in toxicology poses a serious problem compared to a liquid with a more or less dissolved chemical.

Many of the test methods used for evaluation of medical devices are adaptations of established test methods that have been historically used for testing chemicals and pharmaceuticals (see, for example, the OECD guidelines for the testing of chemicals). The adaptations relate to how test samples are prepared from the medical device. In general, medical devices are mixtures of materials, such as polymers, metals and ceramics. While most medical devices are essentially insoluble and solid, some can be soluble solids and liquids. For the insoluble solid devices, test samples are typically prepared by creating an extract of the medical device in various solvents. These solvents are intended to pull out or extract the chemicals that could be anticipated to leach from the device during clinical use. The use of extracts assumes that the *in vitro* extraction procedure results in extraction of those chemicals that will also be released from the medical device during its *in vivo* use in relevant quantities. If extracts of the devices are prepared, the solvents and conditions of extraction used should be appropriate to the nature and use of the final product, as well as to the predictability (such as test purpose, rationale, sensitivity, specificity, etc.) of the test method. To represent polar (aqueous) and non-polar (lipid) fluid environments that a device might be exposed to during clinical use, extractions are accomplished with polar and non-polar solvents. The solvents used and extraction conditions (time and temperature for extraction) are described in ISO 10993-12: 2007 '*Sample preparation and reference materials*'. For the polar solvent, saline is frequently used and for the non-polar solvent, a refined vegetable oil is common. Extract conditions may vary slightly according to the use of the extract (Table 7.2). Once created, the 'device extract' becomes the test solution used for evaluation.

For certain biodegradable materials (e.g. polymers) a so-called exhaustive extraction may be considered, as an alternative to typical extraction methods in ISO 10993-12, as for these materials eventually all chemicals present in the medical device will be released and can thus be a source of potential harm. Methods for such an exhaustive extraction are based on the Japanese Ministry

Table 7.2 Extraction conditions for biological evaluation of medical devices

| Conditions | <i>In vitro</i> cytotoxicity ^a | Other toxicity assays ^b |
|-------------|---|------------------------------------|
| 37°C ± 1°C | 24 h ± 2 h | 72 h ± 2 h |
| 50°C ± 2°C | 72 h ± 2 h | 72 h ± 2 h |
| 70°C ± 2°C | 24 h ± 2 h | 24 h ± 2 h |
| 121°C ± 2°C | 1 h ± 0.2 h | 1 h ± 0.1 h |

^a According to ISO 10993-5:2009.

^b According to ISO 10993-12:2007.

of Health, Labor, and Welfare (MHLW) testing guidelines for medical devices. These extraction methods utilize organic solvents to more aggressively extract leachables from the medical device. The extract is reduced to a residue, which can then be dissolved in a solvent appropriate for the test system. Since the mass of residue is known, the test solution (extract) can be prepared with a defined concentration. In the conventional extraction method, the quantity of leachable in the extract is unknown. It can be argued that the extraction with organic solvents is extreme, does not represent exposure conditions under clinical use, and may result in chemical alterations of possible leachables. However, greater quantities of leachables can be isolated during the exaggerated extraction procedures that might be useful for hazard identification.

For soluble devices, the device is mixed with an appropriate solvent to create a solution at a concentration that is physiologically compatible. With extracts of devices or solutions created from devices, the mixture is typically used immediately since the stability of the solution or extract is unknown. Another difference from test methods with chemicals is that most testing is accomplished using a single dose of the 100% strength extract. This could be considered equivalent to the ‘maximum tolerated dose’ (MTD). This single MTD is used since adverse responses associated with extracts of medical devices are rare.

For some of the test methods, the medical device can be tested directly without the need for extraction. For example, an examination glove would be tested directly in a skin irritation test, and a contact lens solution would be tested directly in an eye irritation test. For the assessment of local effects following implantation (ISO 10993-6), the medical device or parts thereof (or the composing materials) are implanted directly in tissues. Tests to evaluate systemic toxicity may utilize extracts of the device for the acute duration studies and implant portions of the device for subacute, subchronic, and chronic toxicity studies.

7.4 *In vitro* testing

Various *in vitro* assays are included in the ISO 10993 series for the biological evaluation of medical devices. In general *in vitro* assays are useful tools

for identifying potential hazards associated with a chemical compound or material. Both the solid biomaterial and an extract can be used. Only a limited number of *in vitro* tests have been validated for risk assessment purposes, and those mainly for single chemical substances and not for (extracts of) biomaterials or medical devices. To further reduce the use of animals in safety evaluation testing there is a constant need for validation of alternative testing methods including *in vitro* systems. However, some assays are accepted for the purpose of hazard identification, such as *in vitro* genotoxicity assays, whereas *in vitro* irritation assays are mentioned in annexes of existing standards as potential alternatives for animal testing.

A limitation of *in vitro* assays is that they cannot give information on dose response relationships that can be extrapolated to man and thus be used for the final risk assessment of a biomaterial or a medical device (Park *et al.*, 2009). However, an assay such as an *in vitro* cytotoxicity test can be used for screening purposes (is one biomaterial more toxic than another?) and mechanistic studies if some toxicity is encountered (which component of the material is toxic and what is the possible mechanism?). If the material is highly toxic *in vitro*, less toxic components might be used as an alternative to replace the highly toxic ones.

7.4.1 *In vitro* cytotoxicity assays (ISO 10993-5:2009)

The general *in vitro* cytotoxicity assays commonly used for the evaluation of biomaterials/medical devices are described in ISO 10993-5 (ISO 10993-5:2009). The standard includes a schedule for testing leading to a final conclusion as to which test would be most appropriate for the medical device to be evaluated. The assays include three different approaches for the evaluation of cytotoxicity, an extract test, a direct contact test and an indirect contact test. There are numerous *in vitro* cytotoxicity assays available that can be grouped according to the evaluation criteria and read-out system. Cytotoxicity can be determined by evaluation of cell morphology, cell damage, cell growth or by measurement of cellular activity. Various cell lines are available for cytotoxicity testing but established cell lines obtained from a reliable source such as the American Type Culture Collection (ATCC) are preferred.

Read-out systems for cell detection can be neutral red assay, tetrazolium salt assay (e.g. MTT, XTT, WST-1), colony forming assay or the LDH assay detecting primarily membrane damage in cells. However, a reduction in the total amount of LDH in the cell culture (LDH in the supernatant and the remaining cells) can also be indicative for cytotoxicity resulting in a reduced number of cells. A combination of the LDH assay indicating membrane damage with a metabolic activity assay (tetrazolium salt) is commonly used. Membrane damage may be considered as an early signal for cellular cytotoxicity.

In each independent cytotoxicity assay the following controls should be included in addition to the normal cell growth control of the cells cultured in the appropriate tissue culture medium only: a positive control, a negative control and a blank control.

The positive and negative controls should be based on biomaterials/medical devices that are known to induce a cytotoxic or non-cytotoxic response, respectively. For extracts the blank control is important as this is the extraction medium for the preparation of the test samples.

The test sample could be an extract of the material or the material itself. Test samples should be prepared as described in ISO 10993-12:2007 '*Sample preparation and reference materials*'. The extraction conditions, using both a polar and non-polar vehicle, should simulate or exaggerate the expected clinical use situations to identify any potential toxicity (Table 7.2). In general tissue culture medium with serum is preferred, as this supports cell growth and allows for the extraction of both polar and non-polar compounds. During the extraction procedure sterile conditions should be maintained. If this is not possible, for example, if the source material is not sterile, sterilization of the extract and/or test sample should be considered.

For medical devices that have a close, long-term contact with tissues, in addition to extracts, the materials themselves can also be used in a direct contact or indirect contact cytotoxicity assay. This way, the release of chemicals toxic only in close contact or near the biomaterial can be detected. In such cases a toxic effect can only be observed in cells in contact with the material. Seeding cells on a material may not be sufficient for detecting a possible cytotoxicity of the material as the lack of cell growth may be due to the fact that the material by itself does not support cell growth. Toxicity of such materials should be determined on already established cellular monolayers. When a direct contact assay is used, severe general toxicity of the cell culture may be observed due to leakage of highly toxic components from the material (Van Tienhoven *et al.*, 2006).

7.4.2 *In vitro* genotoxicity testing (ISO 10993-3:2003)

Testing for genotoxicity is indicated where a review of the composition of the materials reveals the possible presence in the final medical device of compounds that might interact with genetic material, or when the chemical composition of the medical device is unknown. The genotoxic potential of suspect chemical components should be assessed, bearing in mind the potential for synergy, in preference on the material or medical device as a whole. The genotoxicity testing of a medical device should start with a series of *in vitro* tests. These tests are mainly needed for medical devices with a prolonged (>24 h and ≤ 30 days) or permanent (>30 days) contact time, being external communicating devices or implants (Table 7.1) (ISO 10993-1:2009).

Assays for the detection of genotoxicity are described in ISO 10993-3:2003, which is currently under revision.² The *in vitro* test should include the three critical genotoxicity endpoints; that is, gene mutations, structural and numerical chromosome aberrations.

The induction of gene mutations can be determined in the bacterial reverse mutation test (Ames test) as described in OECD 471, 1997. The bacterial reverse mutation test uses amino acid requiring strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs (OECD 1997). The principle of this bacterial reverse mutation test is that it detects mutations that revert mutations present in the test strains. These induced mutations restore the functional capability of the bacteria to synthesize an essential amino acid resulting in growth on selected media while the non-mutated parent bacteria will not grow on these media.

The induction of gene mutations should also be investigated in mammalian cells. The induction of mutations can be detected by using the *in vitro* mammalian cell gene mutation test (OECD 476) or an *in vitro* MN assay (OECD test guideline 487).

For the *in vitro* gene mutation test several mammalian cell lines can be used that are deficient for thymidine kinase (TK), hypoxanthine-guanine phosphoribosyl transferase (HPRT), or xanthine-guanine phosphoribosyl transferase (XPRT) (OECD 476). The TK deficient mutant cells are able to proliferate in the presence of the pyrimidine analogue trifluorothymidine (TFT), whereas normal cells, which contain thymidine kinase, are not, as they incorporate the pyridine analogue TFT resulting in inhibition of cell division. Similarly, cells deficient in HPRT or XPRT are selected by resistance to 6-thioguanine (TG) or 8-azaguanine (AG). The products of these enzymes are toxic for the cells so, when a mutation occurs, cell growth will be limited by the production of the selective toxic compounds. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency (viability). After a suitable incubation time, colonies are counted. The mutant frequency is derived from the number of mutant colonies in selective medium and the number of colonies in non-selective medium.

In addition to the gene mutation test in mammalian cells, recently an OECD protocol was published describing the *in vitro* MN test (OECD 487).

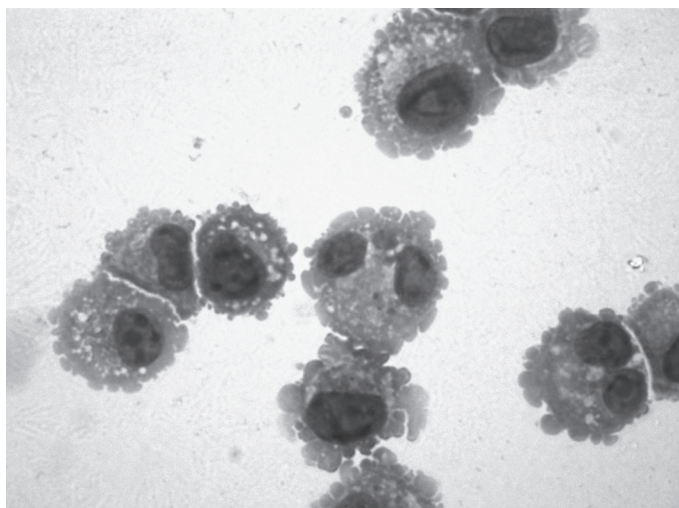
2 Currently ISO 10993-3:2003 Biological evaluation of medical devices - Part 3: Test for genotoxicity, carcinogenicity, and reproductive toxicity, is under revision with the revised standard expected to be published within a few years.

This assay is the counterpart of the *in vivo* MN assay as indicated in ISO 10993-3:2003 (see also section 7.5).

The *in vitro* MN assay is a genotoxicity test for the detection of micronuclei (MN) in the cytoplasm of interphase cells, which can be performed using either rodent or human cells. MN may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic chemicals in cells that have undergone cell division during or after exposure to the test substance. The formation of MN is determined in so-called binucleated cells that are stalled in their cell division by adding cytochalasin B, that inhibits complete cell division by blocking the formation of contractile microfilaments (an example is presented in Fig. 7.3).

The assays can be used with an exogenous source of metabolic activation unless the cells themselves have sufficient metabolizing capability.

When all *in vitro* assays for mutagenicity or DNA damage are negative, further *in vivo* testing is normally not needed. When a positive assay is observed, further *in vivo* testing is required or the presumption has to be made that a material is mutagenic.



7.3 Example of binucleated cell with and without MN. The figure shows A549 human alveolar adenocarcinoma cells *in vitro* treated with cytochalasin B inhibiting cell division thus inducing binucleated cells and a positive genotoxic control bleomycine (5 µg/mL). Note in the centre a binucleated cell with an MN.

7.4.3 Haemocompatibility (ISO 10993-4:2002 and amendment ISO 10993-4:2006)

For medical devices with potential blood contact the possible interaction with various blood components needs to be evaluated. Types of devices with possible blood contact are the non-contact devices (*in vitro* diagnostic devices), the external communicating devices (e.g. cannulae, devices for collection, storage and administration of blood, catheters, guidewires, intra-vascular endoscopes, extracorporeal membrane oxygenators, percutaneous circulatory support systems), and implant devices (e.g. mechanical or tissue heart valves, vascular grafts, stents, arteriovenous shunts, blood monitors, pacemaker leads). Obviously, devices that have no contact with patients (non-contact *in vitro* diagnostic devices) do not need to be investigated for interactions with blood. Also devices with very short-term contact (e.g. scalpels, needles) are generally not evaluated for blood–device interactions. A decision tree, for determination whether specific testing for interactions with blood is indicated, is presented in Fig. 7.4.

Assays evaluating the various possible interactions with blood are described in ISO 10993-4:2002³ and its amendment ISO 10993-4:2006. The following issues need to be considered for medical devices contacting blood: thrombosis, coagulation, platelets, haematology and the complement system (see also Chapter 8). Some but not all of these issues may be approached by using *in vitro* methodologies. *In vitro* tests are regarded as useful in screening external communicating devices or implants, but may not be accurate predictors of blood–device interactions occurring upon prolonged or repeated exposure or permanent contact. However, *in vitro* testing allows for repeating the assays and thus statistical analysis. Also the kinetics of reactions like blood coagulation and thrombus formation can be followed in time. In addition both static and dynamic systems can be used.

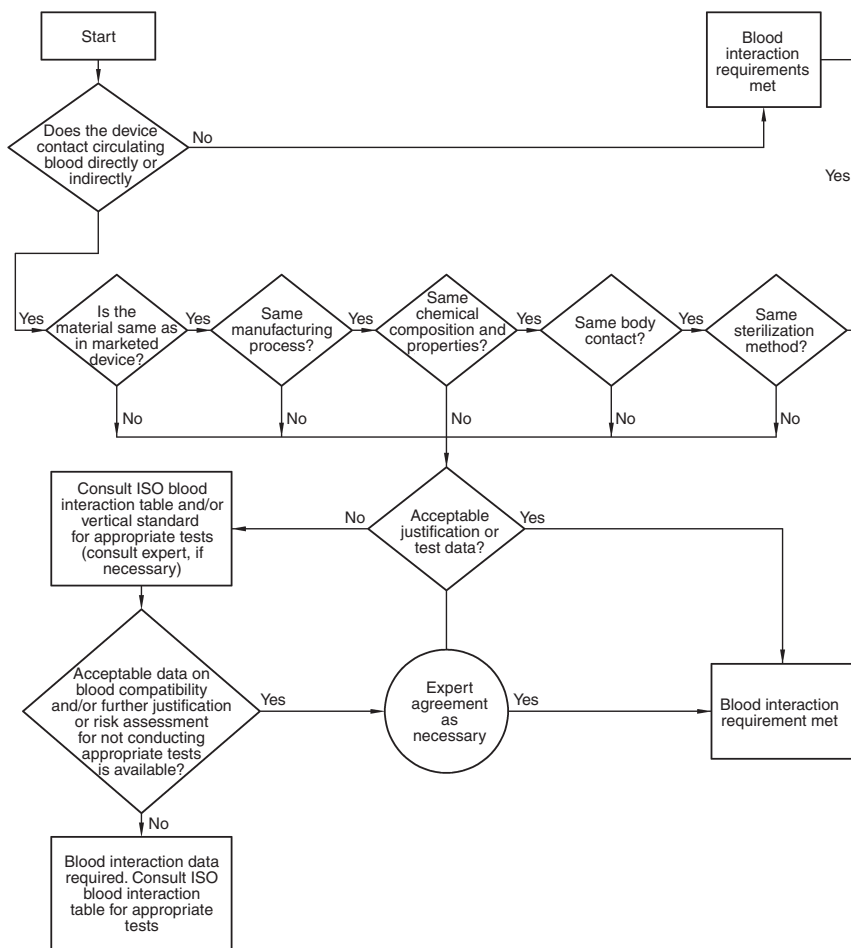
In principle human blood is available and should be used for the *in vitro* testing. However, several limitations may exist for the use of human blood including practical limitations and ethical issues. Various variables exist that may affect the outcome of the test including the use of anticoagulants, sample collection method, sample storage, sample age, aeration and pH, temperature and the testing protocol itself. Storage may decrease the quality of the blood to be used in the assays due to rapid changes of some blood properties. For *in vitro* assays also the laboratory equipment and disposables used should be tested for their interactions with blood to exclude interference with the test outcome.

3 Currently ISO 10993-4:2002 Biological evaluation of medical devices – Part 4: Selection of tests for interactions with blood, is under revision with the revised standard expected to be published within a few years.

It should be realized that many challenges exist for evaluating interactions of blood contact devices by *in vitro* (but also *in vivo*) assay systems, and that no single model will be appropriate for all applications. So, it is critical to consult with vertical product standards if available for specific devices.

Coagulation

Coagulation methods are based on the use of fresh non-anticoagulated whole blood, anticoagulated whole (citrated) blood, platelet rich plasma or platelet poor plasma. Most *in vitro* assays for coagulation are modified



7.4 Decision tree to decide whether testing for interactions with blood is necessary (ISO 10993-4:2002).

existing tests from analytical clinical chemistry, adapted to be used for investigating effects of accelerated coagulation induced by medical devices and/or biomaterials. Various parameters can be determined including partial thromboplastin time (PTT), prothrombin time (PT), thrombin time (TT), thrombin generation, fibrinogen, fibrinogen and fibrin degradation products (FDP), and specific coagulation factors. The most commonly used tests are the thrombin (thrombin-antithrombin complex, T-AT), fibrin (fibrinopeptide A, FPA), and PTT clotting test (ISO 10993-4, revision in preparation). T-AT formation can be measured in the plasma using the ELISA (enzyme-linked immunosorbant assay) methodology. The concentration of this inactive proteinase inhibitor complex (T-AT) gives an indication of the degree of coagulation system activation. High T-AT levels indicate high coagulation (thrombin) activity and low T-AT levels indicate low coagulation activity. High levels of thrombin from a test medical material may indicate a higher tendency for the material to be associated with acute thrombosis.

Platelets

Platelet aggregation can be induced by various aggregation agents and can be determined by using platelet rich plasma (PRP). Activated platelets are pro-thrombogenic. Some materials can activate platelets which can result in the release of platelet granule substances, a change in platelet morphology, and formation of platelet microparticles. The platelet function can be investigated by determining the bleeding time which can also be mimicked by using an *in vitro* filter method. *In vitro* the release of products of the platelet granules like β -TG (beta thromboglobulin) and PF4 (platelet factor 4) can be measured. An enzyme immunoassay (ELISA) method is used for quantitative determination of β -TG released into plasma from platelet alpha granules.

Complement system

There are numerous proteins and protein fragments that make up the complement system and these can be divided into three distinct activation pathways: the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway. It is the alternative complement that is mostly regarded as being affected by and reactive to the presence of medical materials. Various components (e.g. CH 50, C3a, C5a, TCC, Bb, iC3b, C4d, SC5b9) can be evaluated *in vitro*. The most commonly used tests are the determination of C3a as a general indicator for complement activation and SC5b9, which is the soluble form of the terminal membrane attack complex and generally considered a marker representative of the full extent of complement activation (ISO 10993-4, revision in preparation). Both C3a and SC5b9 can be evaluated by the ELISA method.

A disadvantage of measuring the complement fragments *in vitro* is their relatively high background level.

Haemolytic properties

Although haemolysis does seem a simple process there are a number of variables to consider such as contact time, surface characteristics of a material, cell-material interaction, flow level in the test system, and blood source. These variables need to be adequately controlled for comparisons of haemolytic potential among materials and medical devices. Both *in vivo* and *in vitro* simple and more complicated assays have been employed to study haemolysis.

Haemolysis can be determined by measuring *in vitro* damage to erythrocytes either by incubating a medical device/ biomaterial or an extract thereof with erythrocytes. The haemolysis activity can be investigated by measuring the percentage haemoglobin release from the erythrocytes by using one of three classical methods, the cyanmethaemoglobin method, the oxyhaemoglobin method and the iron method. In addition, there are a multitude of different assays available for determining plasma haemoglobin without one of them being widely accepted (ISO 10993-4:2002). Various spectrometric methods to measure free haemoglobin will be included in the currently ongoing revision of ISO 10993-4:2002.

7.4.4 *In vitro* irritation assays (ISO 10993-10:2010)

An *in vitro* irritation assay is included in an Annex of the last revision of ISO 10993-10:2010, the standard describing assays on irritation and skin sensitization. This *in vitro* alternative has been endorsed for chemical substances by ECVAM (European Centre for Validation of Alternative Methods, JRC, Ispra, Italy).

The principle of the *in vitro* skin model irritation assay is based on the premise that irritant chemicals are able to penetrate the stratum corneum by diffusion and are cytotoxic to the cells in the underlying layers. Moreover, if the cytotoxic effect is absent or weak, a quantifiable number of inflammatory mediators are released by the epidermis and may be used in a tiered approach to increase the sensitivity of the test (ISO 10993-10:2010 Annex D).

The *in vitro* skin irritation assays use a reconstructed human-skin model that consists of either commercially available or in house-prepared constructs, consisting of a supporting collagen or other kind of matrix and a functional stratum corneum.

The read-out system for irritation is the viability of the construct after incubation with the test sample and the production of cytokines. The viability is

determined with metabolically converted vital dyes (tetrazolium salts). A negative reference control and a positive reference control should be tested concurrently with the test substances to demonstrate that viability (negative reference control), barrier function and resulting tissue sensitivity (positive reference control) of the tissues are within a defined historical acceptance range.

The most frequently used assay is MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue; CAS number 298-93-1] although other assays also based on the formation of tetrazolium salts can be used as well (e.g. XTT, MTS, WST-1). The test substance is considered to be irritant to skin if in the assay model the tissue viability after exposure and post incubation is below 50%.

Additionally, the production of interleukin-1 α (IL-1 α) may be used as an extra parameter for the identification of irritants. At the moment IL-1 α determination is considered useful as additional information on the irritant potential of chemicals. However, only the results from the MTT assay indicating cell survival are used to designate a test sample an irritant. In the future a combination of the two read-out systems (cell viability and interleukin production) may result in a more reliable prediction of irritant activity of chemicals.

The test samples are incubated with the skin constructs for at least 15 minutes, ensuring sufficient covering of the construct during the incubation. After incubation, the test samples are removed by thorough washing and rinsing with an appropriate buffer or 0.9% NaCl. The tissues are further incubated in fresh medium for 42 h as a post exposure recovery period that allows for recovery from weakly irritant effects, after which period cell survival and IL-1 α production are determined.

Depending on the sensitivity of the test system, in some of the available reconstructed skin models a negative outcome would need confirmation in a rabbit skin irritation test.

7.5 *In vivo* testing

In addition to the *in vitro* testing as described above, *in vivo* testing is also necessary for the safety evaluation of biomaterials and/or medical devices. Some of these *in vivo* tests are needed for confirming or refuting results obtained in the *in vitro* assays (e.g. genotoxicity responses), whereas other toxicological endpoints may only be determined by *in vivo* testing (e.g. local tissue reactions, sensitization).

7.5.1 *In vivo* genotoxicity (ISO 10993-3:2003)

Under the testing recommendations of ISO 10993-3, *in vivo* genotoxicity testing is not required unless a genotoxic response is noted in one of the *in vitro* assays for the device. As a note, some countries do not recognize ISO

10993-3 as a consensus standard and require *in vivo* genotoxicity assays as part of the overall genotoxicity assessment regardless of the response in the *in vitro* assays. When *in vivo* genotoxicity assays are required, *in vivo* tests for chromosomal damage in rodent haematopoietic cells are typically used. The two most common assays in this category are: (1) the *in vivo* mouse MN assays and (2) the *in vivo* chromosomal aberration assay.

To expose animals to the medical device in these assays, if the device is soluble, a solution is prepared. For insoluble devices, the device is incubated in solvents in order to extract or remove chemicals. Depending on the regulatory body the data is being submitted to, exaggerated extraction methods may be required for devices composed of polymeric materials.

For classical *in vivo* genotoxicity assays, animals are dosed with a known quantity of a chemical at multiple dose levels where the highest dose produces little or no toxicity. Alternatively for a non-toxic chemical, a single dose or limit dose can be administered at 2000 mg/kg. With soluble medical devices, the same dose selection strategy can be applied. With standard extraction methods for insoluble medical devices, the quantity of chemicals in the extract is unknown, and the dose administered is based on the maximum volume recommended for the assay. In the case of both the mouse MN and *in vivo* chromosomal aberration assays, this maximum dose volume is 20 mL/kg. When the exaggerated extraction methods are used and sufficient quantities of extractable material are obtained, the resulting residue can be administered as a known dose (mg/kg). Depending on the toxicity of the solution obtained from the device, a limit type dose may be appropriate.

In vivo mouse micronucleus assays

The *in vivo* mouse MN assay currently is the more popular of the two *in vivo* assays mentioned. The basic methodology is based on OECD 474, Mammalian Erythrocyte Micronucleus Test. The assay is conducted in young rodents, either mice or rats, but mice are used most commonly. Assays are conducted with concurrent negative (vehicle) and positive controls. A minimum of five males and five females per group are used. Both sexes are used unless a single sex is justified. Animals are dosed with the device extract or solution by an appropriate route. The intravenous route is typically used for saline extracts (insoluble devices) and the intraperitoneal route for other extracts and solutions. The oral route of administration may be applicable for devices that have exposure through the gastrointestinal system. If bone marrow is used, the animals are sacrificed at appropriate times after treatment, the bone marrow extracted, and preparations made and stained. When peripheral blood is used, the blood is collected at appropriate times after treatment and smear preparations are made and stained. For studies with peripheral blood, smears can be prepared as with bone marrow specimens or samples can be analysed by flow cytometry. With both methods,

preparations are analysed for the presence of MN. Numbers of MN in test animals are compared to the negative control to determine whether the treatment caused an increase in MN.

In vivo chromosomal aberration assay

The basic methodology is based on OECD 475, Mammalian Bone Marrow Chromosome Aberration Test. As with the mouse MN assay, the test is conducted in young rodents with similar numbers and groups. Animals are dosed once daily for two consecutive days (multiple or split dosing may be justified) and the dose is based on a maximum volume per kg body weight or mg/kg with extract residues. Animals are sacrificed at 1.5 cell cycle hour times after the last treatment, which is approximately 12–18 h for mice. Prior to sacrifice (3–5 h for mice), animals are treated with a metaphase-arresting agent (Colchicine). Bone marrow cells are harvested; slides are prepared and metaphase cells are scored for different types of chromosomal aberrations. The percentage of metaphase cells with aberrations in the test group is compared to the negative control to determine whether the treatment caused an increase in aberrations.

7.5.2 Carcinogenicity (ISO 10993-3:2003)

Carcinogenicity testing is usually not required for medical devices. The situations where carcinogenicity testing may be required are: (1) a positive response in a genotoxicity assay, (2) the known presence of a potential genotoxic or carcinogenic material within the device, (3) a degradable device with a degradation or absorption time of greater than 30 days and without an adequate history of safe human use, and (4) any biomaterial or device introduced into the body or its cavities with a permanent or cumulative contact of greater than 30 days and without an adequate safe human-use history.

When testing to address carcinogenicity is necessary, it is accomplished using one of two methods: (1) chronic or lifelong studies in rodents or (2) studies in transgenic rodents. Chronic or lifelong studies in mice or rats are based on OECD 451, Carcinogenicity Studies, or OECD 453, Combined Chronic Toxicity/Carcinogenicity Studies. Study durations are usually at least 18 months for mice and 24 months for rats. The study design would have a negative control and test groups with a recommended group size of ≥ 50 animals per sex per group. At the end of the study for a valid negative response, $>50\%$ of the animals in the negative and test groups should have survived. The tumour incidence along with tumour type is analysed between treatment groups to determine if the test article increased the incidence of any tumours. Besides being a very expensive and long duration study, rodents are prone to development of sarcoma surrounding implanted

foreign materials. The development of tumours around implanted materials begins to occur in 8–9 months following implantation and is known as the Oppenheimer effect (Oppenheimer *et al.*, 1956). This is a confounding factor for conducting long-term device implant studies in rodents.

As an alternative to lifelong studies, carcinogenicity studies can be conducted in transgenic rodent models. The most commonly used transgenic model for device carcinogenicity testing is the rasH2 mouse model. The rasH2 mouse model carries the human c-Ha-ras oncogene in addition to the endogenous murine Ha-ras oncogene. This genetic manipulation causes these mice to be very susceptible to tumour development following exposure to carcinogens. With this model, studies are six months in duration rather than 18–24 months. Additionally, since the studies are shorter in duration, the Oppenheimer effect is not an issue. The design of these studies utilizes 20–25 mice per sex per group. Negative and positive control groups are typically included. Mice are implanted with representative portions of the device. The amount implanted should represent a significant safety factor or exaggeration of the human clinical dose. A 100 × safety factor or greater should be considered, but the amount of material implanted must be physiologically compatible for implantation in mice. Implanted portions of device should be no greater than 15 mm across and 2–3 mm thick with rounded edges. Multiple samples this size may be implanted to achieve the desired dose. As with lifelong studies, the tumour incidence and types are compared statistically between the treatment groups.

7.5.3 Haemocompatibility (ISO 10993-4:2002 and amendment ISO 10993-4:2006)

As stated earlier in this chapter, effects on thrombosis, coagulation, platelets, haematology, and the complement system must be considered for medical devices contacting blood (see also the decision tree as presented in Fig. 7.4). Many of these aspects of haemocompatibility can be addressed through *in vitro* test methods. These test methods were discussed previously in this chapter and in the following Chapter 8, 'Blood compatibility assessment in medical devices: considerations and standards'. *In vivo* haemocompatibility tests are most often used to address thrombosis, local tissue responses, and efficacy of the device, frequently utilizing the device under conditions mimicking clinical use.

In vitro assays are useful in evaluating basic haemocompatibility properties of biomaterials. The complicated geometry and physical characteristics of a device can influence the potential for thrombosis, or thrombogenicity. Consequently, evaluations utilizing *in vivo* models to assess thrombosis in the vasculature with flowing blood are frequently necessary. The devices

used for these tests should be finished devices to reflect not only the bio-materials, but also the shape and surface characteristics of the device. For this reason, large animal models are typically needed due to the size of the devices. Historically, canines, swine and sheep have most commonly been used as models. These animal models have blood vessels and cardiovascular anatomy of sufficient size that allows for utilization of human-size devices. However, the blood vessels in these models are usually smaller than those in humans.

As a model, the cardiovascular system and blood of swine are considered very similar to humans. Blood vessels in swine are proportionately smaller than those in humans on a body-weight basis. Peripheral vessels in swine tend to be deeper (less accessible) and more prone to vasospasm, making device insertion more challenging. The peripheral vessels in canines, while proportionately large on a body-weight basis, are still smaller than human vessels. Canines are easy to work with and the blood vessels are more accessible, but canines have been considered more prone to coagulation (hypercoagulopathy) (Bruck, 1977). This increased coagulation potential could be viewed as preferred, since these models are used for purposes of safety assessments. Sheep have been used as an animal model due to the similarity of their coagulation profiles to humans. They are the primary model for the evaluation of heart valves (Siller *et al.*, 2008, Byrom *et al.*, 2010).

In vivo thrombosis testing

This test is typically conducted to evaluate acute thrombosis associated with devices that are placed in circulating blood. The device may have limited, prolonged or permanent exposure. To address thrombosis at subacute, subchronic or chronic durations, thrombosis may be addressed as an end-point within an efficacy type implant study. There is no standardized test method to evaluate acute thrombosis. This is due to the fact that finished cardiovascular devices are used and conditions of clinical use can vary widely. Thrombosis with a device is affected by the location of use (arterial or venous and vessel diameter), duration of exposure, and use of anticoagulants. Applying a single test method to all devices may result in an over- or under-challenge as compared to the conditions of clinical use. However, a 4 h, non-heparinized, venous implant model has been suggested by some as a 'standard model'. This model may be appropriate for various catheters and guidewires.

This test is typically conducted in dogs, pigs or sheep, with dogs historically being used most often. Due to concerns about dog use, there is a shift from dogs to farm animals. A minimum of three animals is recommended. The jugular vein is commonly used as an implant site due to its relatively large size, length, straightness and accessibility. As a paired vessel, a test

Table 7.3 Subjective thrombosis scoring scheme (ISO 10993-4:2002)

| Score | Description |
|-------|--|
| 0 | No thrombosis (small clot at insertion point possible) |
| 1 | Minimal thrombosis, e.g. at one location or very thin layer |
| 2 | Slight thrombosis, e.g. minimal clotting at multiple locations |
| 3 | Moderate thrombosis, e.g. less than 1/2 length of implant covered with clot |
| 4 | Severe thrombosis, e.g. greater than 1/2 length of implant covered with clot |
| 5 | Vessel occluded |

device can be placed in one jugular vein and a comparative control in the opposite vein. The comparative control should be a similar device or clinically equivalent to the one being tested. The control should have a safe history of use with respect to thrombosis. Since there is no defined acceptable or unacceptable amount of thrombosis, having an ‘accepted’ control provides a reference to relate and compare thrombus formation.

Animals are anaesthetized and the implant site receives a surgical preparation. The jugular veins are surgically exposed and a portion of the device is inserted into the vein at the cranial aspect of the neck. The device is threaded down the vessel toward the heart for a distance of approximately 15 cm. This is usually a sufficient length to allow for device evaluation, while staying with the straight portion of the jugular vein. The device is sutured in place at the insertion point to prevent blood leakage and movement. The device should not prevent or stagnate blood flow within the vessel. Animals are maintained under anesthesia during the 4-h implant period. At the end of the implant period, animals are heparinized to prevent post-mortem clotting. After sufficient time for heparinization, animals are euthanized and exsanguinated. Vessels are removed with devices in place. The vessels are opened and the device and vessel lumen/wall scored for the presence of thrombus formation. Most scoring schemes are subjective as shown in Table 7.3. Besides subjective assessments, clots can be weighed to further quantify the amount of thrombus present

7.5.4 Implantation (ISO 10993-6:2007)

For devices placed within tissues, the ISO 10993-1 standard requires that the local pathological effects on living tissue from implanted devices be assessed. This assessment is accomplished through gross and microscopic examination of tissues. Samples of the device or biomaterial are surgically implanted in an appropriate tissue based on the clinical use. Common tissues are muscle, subcutaneous tissue and bone, but other specific tissues, such as brain, ocular and dental tissue, may be appropriate based on the intended clinical use. Since tissue responses change over time and considering absorption of

degradable materials, evaluations of the local responses at multiple implant durations are typically required. For permanent implants, implant intervals should encompass short-term and long-term intervals. Ideally, the long-term interval should be sufficient that the local tissue response has reached homeostasis or steady state. With non-degradable materials steady state is typically reached in approximately 12 weeks in soft tissues, but can take as long as 26 weeks in bone. For degradable materials, the intervals should be carried out to the point of complete material degradation, resorption and tissue restoration. For slowly degrading materials a predegradation process *in vitro* might be used to reduce the evaluation period (de Jong *et al.*, 2005). Short-term intervals are typically considered 1–4 weeks in duration, although two weeks as an early interval is preferred as changes associated with surgical trauma will be the predominate finding prior to two weeks. Long-term intervals typically range from 12 to 56 weeks. In implantation studies, control materials are implanted for comparison. Since any implanted material will elicit some response, it is necessary to compare test materials to negative control materials with well-known accepted local reactions, for example (certified) high density polyethylene. The macroscopic assessment is based on the zone of tissue response and/or encapsulation surrounding the implanted specimen. For the microscopic evaluation, implant sites are scored based on the inflammatory cells that have migrated to the site (number per high powered field and width of zone surrounding the implant), presence of necrosis, fibrosis, vascularization, fatty infiltration and other tissue alterations. In some scoring schemes, the reaction for the control material is subtracted from the reaction of the test material. The resultant score is correlated to a scale and defined as slight to severe irritant.

Influence of shape and characteristics

As stated earlier, any foreign material in a tissue will elicit some amount of cellular response resulting in implant encapsulation. This response can be influenced by the material's physical characteristics. For example, a specimen of high density polyethylene as a solid, smooth sheet will elicit a minimal tissue response in muscle. However, implant the same polyethylene as a highly porous mesh, and the local tissue response will be more severe. This difference is primarily attributable to the much larger surface area of the mesh. The fact that the response is more severe is not necessarily a bad outcome. If the mesh is intended for hernia repair, this increased response may be associated with fibrosis and tissue growth, which is for such a device a desired response as part of the healing process.

During the preparation of samples for implantation, it is important to minimize sharp edges and corners as these can cause mechanical trauma and may result in an increased inflammatory response (Wood *et al.*, 1970;

Matlaga *et al.*, 1976). Since physical characteristics, for example, size, shape, form, degradation, etc., will affect the response, it is important to select a control that is similar to the test material being implanted. For example, a collagen sponge used for haemostasis may be expected to be fully absorbed within one month following implantation. Since this material is degradable, it will cause an influx of macrophages, polymorphonuclear cells, and giant cells to come into the site to remove this material. If a specimen of a non-degradable inert material, for example, high density polyethylene, is implanted as the control, there is nothing to be absorbed and the local response to this material will be much less. Consequently, the degradable material may be scored as an irritant based on standard scoring schemes. However, if a comparable material was included as an equivalent or comparative control, this degradable material would provide a better material for comparison. So, when planning implantation studies, it is critical to select appropriate control materials for comparison.

Rabbit muscle implantation study

Muscle as a tissue is frequently used as a surrogate for a variety of tissues since it is highly vascular and the response in muscle is similar to other soft tissues. Subcutaneous tissue may be used as an alternative when the size of an implanted specimen is a limitation for muscle site, that is, larger specimens can be implanted in subcutaneous spaces. The rabbit muscle implantation study is a commonly used model for evaluation of the local tissue response to materials. Rabbits have large paravertebral muscles on each side of the spine that provide a large uniform site for implantation of materials. A typical rabbit implant study uses three rabbits. For longer duration intervals, the number is increased due to the potential for animal loss. Sufficient specimens are implanted such that at the end of the study, at least ten test and ten control sites are available for evaluation. Additional sites are implanted since some sites may not be located or be suitable for evaluation. Specimens are implanted by one of two methods: trochar or surgical. In the trochar method, specimens are implanted through a large gauge needle, that is, 16 gauge. For this method to be utilized, the material must be such that it can easily be cut into a shape that will fit within a needle, that is, 1 mm x 1 mm x 10 mm. Once the needle with biomaterial loaded is inserted within the muscle tissue, a stylet is used to push the material out of the needle and into the muscle. Typically 4–6 test specimens are implanted at one side and a similar number of controls specimens on the opposite side. For the surgical method, small pockets are created along the paravertebral muscle for 4–6 implant sites per side. Once the implant material is placed in the muscle, it is closed with sutures. With degradable and absorbable materials, a non-degradable location marker is placed at the implantation site to aid in identifying the

site at a later date. The location marker is usually a small specimen of the negative control material. At the end of the implantation period, animals are euthanized and muscle removed from the carcass. Muscle may be cut immediately to examine sites macroscopically, but often they are allowed to fix in 10% neutral buffered formalin (NBF) for several days prior to sectioning and examination. The muscle sites are methodologically sectioned, and all implant sites identified. Local tissue responses, that is, encapsulation, haemorrhage, exudates, etc. are scored and described. Sites are histologically processed and microscopic slides evaluated. Sites are scored microscopically for cellular infiltrates, necrosis and other tissue reactions. The mean responses for the test sites are compared to the controls.

Implant studies as part of other studies

When addressing local effects following implantation, these data may be obtained from other studies where the primary endpoint is not local effects. For example, the route of exposure for subchronic and chronic toxicity testing is usually implantation of a device. At the end of the toxicity study, these implant sites are also evaluated, providing local effects data at these intervals. Additionally, studies conducted for functional or efficacy purposes with implantable devices can provide tissues to evaluate local effects.

7.5.5 Irritation (ISO 10993-10:2010)

Irritation is a biological effect that must be addressed for essentially all medical devices. Irritation tests provide an assessment of the local inflammatory tissue response following direct contact with a medical device or extract of a device. As with other tests described, these tests are classic test models that have been historically used for chemicals and have been adapted for medical devices. As described early in this chapter, several *in vitro* alternatives have been developed. These tests are sensitive and they can be used to identify irritants. However, current test guidelines require confirmation of a negative *in vitro* result with an *in vivo* assay. Since the majority of devices tested are non-irritants, widespread use of *in vitro* irritation assays may not occur until regulatory agencies change their position on these assays.

There are a wide variety of irritation assays used to evaluate medical devices. They include skin, ocular, mucous membrane and intracutaneous assays. The choice in the type of irritation assay to select is based on the type of tissue contact the device has with the body. Table 7.4 provides examples of the type test for a given device. For surface contacting devices the irritation assays used reflect the clinical tissue contact. For implanted devices, the intracutaneous reactivity assay is used unless a rationale exists for a more appropriate assay. Irritation assays are typically short-term, with

Table 7.4 Examples of devices and irritation assays (ISO 10993-10:2010)

| Device | Tissue contact | Assay |
|-------------------------|--------------------------------|---|
| Apnoea mask | Skin | Primary skin irritation assay |
| Urinary catheter | Uro-genital mucous membrane | Bladder or vaginal irritation assay |
| Dental appliance | Oral mucous membranes | Oral mucosa irritation test, i.e. hamster cheek pouch assay |
| Lens rewetting solution | Ocular mucous membranes/tissue | Ocular irritation test |
| Orthopaedic implant | Tissue/bone | Intracutaneous reactivity assay |

the visual assessments of observations such as oedema and erythema being compared between test and control conditions. As with many assays, the final evaluation of results is often based on a comparison to the control condition. The following paragraphs present some of the more common irritation assays.

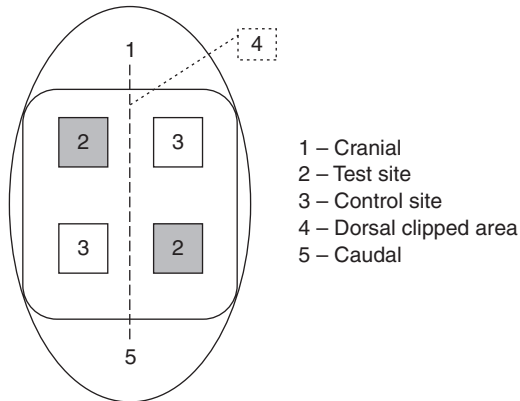
Skin irritation

The New Zealand White (NZW) rabbit is a long-standing model used to assess skin irritation, because it is easy to assess skin reactions in this albino animal model. Three young rabbits weighing at least 2 kg are used for this assay. The fur over the back and sides is removed with electric clippers. Animals are exposed by applying extracts to 2.5 cm × 2.5 cm gauze patches, and placing these on each side of the back (Fig. 7.5).

The patches are covered with an occlusive dressing and secured to the skin. Patches remain in skin contact for a minimum of 4 h, but 24 h is typically used. At the end of the exposure period, the dressings and patches are removed and the skin is washed and dried. The sites are graded for erythema and oedema at 1, 24, 48 and 72 h after patch removal using the standardized Draize Dermal Irritation Scoring scale (Table 7.5).

The 1-h score is not used in the evaluation as the skin reaction at this point may reflect transitory, mechanical effects from presence of the gauze and dressing. The erythema and oedema scores from 24, 48, and 72 h are combined and averaged for the test and control sites. The mean control score is subtracted from the test score. The resultant score is the Primary Irritation Index (PII). Based on the value of the PII, the test article is placed in an irritation (response) category (Table 7.6).

Solid materials such as gloves, wound dressing and other flat materials can be applied directly to the skin. These materials are moistened with water or other appropriate solvents to ensure good skin contact. Testing procedures are otherwise the same as described for extracts.



7.5 Location of skin application sites for irritation testing (ISO 10993-10:2010).

Table 7.5 Skin reaction scoring scheme (ISO 10993-10:2010)

| Reaction | Irritation score |
|---|------------------|
| Erythema and eschar formation | |
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate erythema | 3 |
| Severe erythema (beet-redness) to eschar formation preventing grading of erythema | 4 |
| Oedema formation | |
| No oedema | 0 |
| Very slight oedema (barely perceptible) | 1 |
| Well-defined oedema (edges of area well-defined by definite raising) | 2 |
| Moderate oedema (raised approximately 1 mm) | 3 |
| Severe oedema (raised more than 1 mm and extending beyond exposure area) | 4 |
| Maximal possible score for irritation | 8 |

Table 7.6 Primary irritation index categories in a rabbit (ISO 10993-10:2010)

| Mean score | Response category |
|------------|-------------------|
| 0 to 0.4 | Negligible |
| 0.5 to 1.9 | Slight |
| 2 to 4.9 | Moderate |
| 5 to 8 | Severe |

Ocular irritation

The NZW rabbit has historically been used to assess ocular irritation, because it is easy to assess ocular reactions due to the lack of ocular pigmentation. The test methods are based on OECD 405 and U.S. Consumer Product Safety Commission test guidelines. Three young rabbits weighing at least 2 kg are typically used for this assay. Any material previously shown to be a skin irritant or having a $\text{pH} \leq 2.0$ or ≥ 11.5 should not be tested and is assumed to be an ocular irritant. For materials with insufficient data to make an assessment of possible ocular irritation, an initial screen must be conducted in a single animal. Positive evidence of ocular irritation allows the material to be labelled as an ocular irritant with no further testing. However, if no significant response is observed, testing continues in the remaining animals.

Test methods involve placing 0.1 mL of the test extract or solution in the lower conjunctival sac of one eye in each rabbit. The eyelid is held closed for one second. The opposite eye is similarly treated with the control vehicle or solution. The standard test method involves a single application and observations for three days. However, based on the nature of the clinical exposure, repeat applications and longer durations may be appropriate. Eyes are observed and scored for ocular reactions based on a standardized scoring system (Table 7.7) at 1, 24, 48 and 72 h after treatment. Animals showing severe reactions are humanely euthanized.

Results in the test-treated eyes are compared to the control eyes. If more than one test-treated eye has a positive response in any of the categories, the material is considered an eye irritant. If only one test-treated eye demonstrates a positive response, the results are considered equivocal and the test is repeated in additional animals. A severe reaction in one animal is considered sufficient to consider the material an irritant.

Mucosal irritation

For devices that contact mucous membranes there are a variety of assays, specific for the type of tissue exposure, that is, oral, penile, rectal and vaginal. These assays are less common and do not have OECD equivalent methods. However, they have long histories of use for evaluation of dental materials and topical pharmaceuticals. Each method is similar in that it involves application of the material to mucosal surface, visual observations of any tissue response, and microscopic evaluation of the tissues. Microscopic evaluation of the tissue is the primary basis of evaluation. Tissues are evaluated using a semi-quantitative scoring scheme where epithelial changes, leukocyte infiltration, congestion and oedema are evaluated. The scores for the test are compared to the control, and based on the difference an 'irritation index' is assigned.

Table 7.7 System for grading ocular lesions (ISO 10993-10:2010)

| Reaction | Numerical grading |
|---|-------------------|
| 1. Cornea | |
| Degree of opacity (most dense area) | |
| No opacity | 0 |
| Scattered or diffuse areas, details of iris clearly visible | 1 ^a |
| Easily discernible translucent areas, details of iris slightly obscured | 2 ^a |
| Opalescent areas, no details of iris visible, size of pupil barely discernible | 3 ^a |
| Opaque, details of iris not visible | 4 ^a |
| Area of cornea involved | |
| One-quarter (or less), not zero | 0 |
| Greater than one-quarter, but less than half | 1 |
| Greater than half, but less than three-quarters | 2 |
| Greater than three-quarters, up to whole area | 3 |
| 2. Iris | |
| Normal | 0 |
| Folds above normal, congestion swelling, circumcorneal injection (any or all or a combination of these), iris still reacting to light (sluggish reaction is positive) | 1 ^a |
| No reaction to light, haemorrhage, gross destruction (any or all of these) | 2 ^a |
| 3. Conjunctivae | |
| Redness (refers to palpebral and bulbar conjunctiva excluding cornea and iris) | |
| Vessels normal | 0 |
| Vessels definitely injected above normal | 1 |
| More diffuse, deeper crimson red, individual vessels not easily discernable | 2 ^a |
| Diffuse beefy red | 3 ^a |
| Chemosis | |
| No swelling | 0 |
| Any swelling above normal (including nictitating membrane) | 1 |
| Obvious swelling with partial eversion of lids | 2 ^a |
| Swelling with lids about half-closed | 3 ^a |
| Swelling with lids about half-closed to completely closed | 4 ^a |
| Discharge | |
| No discharge | 0 |
| Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) | 1 |
| Discharge with moistening of the lids and hairs just adjacent to lids | 2 |
| Discharge with moistening of the lids and hairs, and considerable area around the eye | 3 |

^a Positive result

While these tests can be conducted in a variety of small animal models, the oral irritation is frequently conducted using the Chinese Syrian Hamster. This model has been historically used for evaluation of dental materials (Roy and Wishe 1986; Harsanyi *et al.*, 1991). In this model, solid materials in the shape of a disc are placed inside the cheek pouch or for liquids a cotton-wool pellet is soaked with the test liquid. The test sample is placed in one cheek pouch and the control material in the opposite pouch of ten animals. A collar is placed around the neck to maintain the sample within the cheek pouch. At the end of 7–14 days, animals are euthanized and the cheek pouch is visually inspected and collected for histopathology. Tissues are evaluated and an irritation index assigned.

For the penile, rectal, and vaginal irritation assays, rabbits are typically used. As an animal model for the vaginal irritation assay, the rabbit vaginal mucosa is considered more sensitive to irritants than humans (Eckstein *et al.*, 1969; Kaminsky *et al.*, 1985). As a result, it may overestimate the irritation potential of a material in humans. For each study separate test and control animals are used, with a minimum of three animals per group utilized. In the penile irritation, the prepuce is reflected and the penis coated with approximately 0.2 mL of test or control material. This treatment is repeated hourly for four treatments. The genital tissue is examined for any local irritation at 24 and 48 h following the last treatment. Following the 48 h examination, animals are euthanized and tissues collected for histopathology. Tissues are evaluated and an irritation index assigned. In the rectal and vaginal irritation assay, a catheter is inserted into the rectum or vagina and a 1 mL dose delivered. Animals are dosed daily for five days with daily observations of the local tissues. At 24 h following the last dose, animals are euthanized and tissues collected for histopathology. Tissues are evaluated and an irritation index assigned.

Intracutaneous reactivity

The intracutaneous reactivity test is a standard screening assay for medical devices regardless of their tissue contact during clinical use. This test has a long history of use as part of the United States Pharmacopeia (USP) method for evaluating leachables associated with pharmaceutical containers. In the USP version of the test, four extracts are used: saline, 5% alcohol saline, propylene glycol and vegetable oil. Today, for most medical devices, only saline and vegetable oil extracts are evaluated. As with the skin irritation test, materials with a $\text{pH} \leq 2.0$ or ≥ 11.5 are assumed to be irritants and not tested. In this model, albino (NZW) rabbits are used. The current ISO version requires three rabbits weighing at least 2.0 kg. Following closely clipping the fur over the back, 0.2 mL of test and control extracts are injected intradermally with a small gauge needle at five sites. Extracts are

injected in rows. In each rabbit a row of five test saline blebs and five saline control blebs will be placed on one side with the vegetable oil injections place on the opposite side. The appearance of the blebs is evaluated for erythema and oedema immediately after injection, and at 24, 48 and 72 h following injection using a standardized scoring scheme (Table 7.5). Scores for erythema and oedema for all sites and animals are added and divided by the total observations to arrive at an average irritation score for the test and control extracts. The value of the respective control is subtracted from the test extract score. The extract meets the criteria of the test if the value is ≤ 1.0 . An important technical consideration regarding this assay is the use of high quality vegetable oil. If the vegetable oil used is not refined or has become rancid, it will cause excessive reactivity obscuring a reaction associated with leachables. With refined vegetable oil, scores of 1 for erythema and possibly oedema are common at 24 and 48 h but are reduced or absent by 72 h. Scores of ≥ 2 , particularly for erythema with the vehicle control may suggest an issue with the oil.

7.5.6 Sensitization (ISO 10993-10:2010)

Evaluation of a medical device's potential to cause sensitization is required for all devices regardless of tissue contact and contact duration. The tests used are classical models that have a long history of use. Currently, there are three basic models, 1) the guinea pig maximization test (GPMT), 2) the guinea pig closed patch or Buehler model, and 3) the local lymph node assay (LLNA). The GPMT is used most frequently and can be used regardless of the device's tissue contact. The Buehler method is typically reserved for devices that only have contact with intact skin. The two guinea pig test methods are qualitative assays, while the LLNA is a quantitative method. The LLNA has a long history of use for chemicals, but has a relatively short history of use in medical device testing. Due to less historical data with this assay for medical devices, some regulatory agencies do not fully recognize this method at this time.

Guinea pig maximization test

The basic methodology is described in ISO 10993-10 and similar to other standard methods such as OECD 406, Skin Sensitization. The test is conducted in young guinea pigs weighing 300–500 g at the beginning of the test. Either sex can be used, but all should be the same sex, and females should not have been or currently be pregnant. The test utilizes ten animals per test extract with five animals for the respective control extract. So for a

study with saline (polar) and non-polar (vegetable oil) extracts, 30 animals are used. ISO 10993-10 requires that positive control materials be tested at least once every six months utilizing a weak sensitizer. The study has three phases: intradermal induction, topical induction and challenge. For the intradermal induction animals receive three pairs of intradermal injections over the scapular region. The pairs are as follows:

Site A – a 50:50 mixture of the chosen solvent/extract vehicle and Freund's complete adjuvant. The mixture is well mixed to form a stable emulsion.

Site B – the test sample (undiluted extract); inject the control animals with the solvent alone.

Site C – a 50:50 mixture of the test extract/solution and Freund's complete adjuvant. The mixture is well mixed to form a stable emulsion. Control animals are injected with a 50:50 mixture of the respective solvent/extract vehicle and Freund's complete adjuvant.

Topical induction occurs approximately one week after the intradermal injections. One day prior to topical induction, the previous injection site area is clipped to remove excess hair and approximately 0.5 mL of 10% sodium lauryl sulphate (SLS) in petroleum is applied to the area to cause mild irritation that will improve topical absorption. Twenty-four hours after application of the SLS, it is removed. The test extract/solution is applied to an approximate 8 cm² filter paper or absorbant gauze, then applied over the previous injection site area and covered with an occlusive dressing. The patches are removed after 48 h. Two weeks after this topical induction, hair is closely clipped over the back and flank region. In the test animals, the test extract/solution is applied to the right flank and the vehicle control is applied to the left flank. In the control animals, the control vehicle/solution is applied to the right flank and the test applied to the left. Test and control materials are left in place for 24 h, then removed and sites are scored for erythema and oedema using a standardized scale (Table 7.8) at 24 and 48 h following patch removal.

In general, scores of 1 or greater are considered evidence of sensitization. However, scores of 1 or greater may also be seen in the control animals. Responses greater than the control are considered a positive indication of

Table 7.8 Magnussen and Kligman (ISO 10993-10:2010)

| Patch test reaction | Grading scale |
|----------------------------------|---------------|
| No visible change | 0 |
| Discrete or patchy erythema | 1 |
| Moderate and confluent erythema | 2 |
| Intense erythema and/or swelling | 3 |

sensitization. The overall response in the test animals is compared to the response in the control animals. In the situation of an equivocal response, animals should receive a rechallenge of the topical application. This is accomplished 1–2 weeks following the initial challenge and the materials are applied to fresh skin sites.

Guinea pig closed patch test

The basic methodology is described in ISO 10993-10 and similar to other standard methods such as OECD 406, Skin Sensitization. Animal requirements and numbers of animals are the same as with the GPMT. This method is typically used for materials having contact with intact skin and when a part of the device can be applied directly to the skin of the guinea pig, for example, a glove, drape, bandage material, electrodes, etc. While extracts of a device may be used, this situation is less common. In the closed patch method, after clipping the hair, a 25 mm × 25 mm section of the test article is applied to animals for a 6–8 h period, three times per week for a three-week period. With chemicals, the application is usually once per week. However, with medical devices and the low concentration of potential sensitizers, the frequency of application during induction is increased to improve the detection capability of the assay. In the control animals, a negative control material such as cotton gauze is used. After the three-week induction period, there is a two-week rest period followed by clipping the hair and applying a topical challenge (tenth application of the test or control article). As with the GPMT, test and control animals are patched with both test and control articles. Scoring for erythema and oedema is the same as with the GPMT. Evaluation procedures are the same as the GPMT.

Local lymph node assay

The LLNA was validated as an alternative test method to determine the sensitization potential of individual chemicals in 1999. In 2002, it was accepted by OECD as OECD 429 – Skin Sensitisation: Local Lymph Node Assay. After several years, this method was adopted for use with medical device extracts. The current version of ISO 10993-10 contains detailed methods for the conduct of this assay. The assay offers several advantages over the guinea pig sensitization assays. It is a quantitative assay, can be conducted in less than a week, needs relatively small amounts of material for testing, and has animal welfare benefits. The disadvantage is that in recent years there have been questions about: (1) the validity of using this assay with extracts that are by their nature mixtures as against single chemicals, and (2) whether the method is appropriate with aqueous solvents. Due to these questions, some regulatory agencies have not recommended this assay. However, new data

have been presented recently supporting the LLNA as valid with mixtures and an aqueous solvent.

Non-polar or hydrophobic solvents have been historically used with the LLNA for chemicals. These solvents are readily absorbed by the skin. Aqueous solvents tend to bead up and roll off the skin. To utilize aqueous medical device extracts it was necessary to improve skin adherence and absorption. This has been accomplished in one of two methods. The first is through the addition of a thickener such as carboxy methyl cellulose or hydroxyethyl cellulose (0.5% w/v). Alternatively, the use of a surfactant such as 1% pluronic has been recommended (Ryan *et al.*, 2002). The addition of either the thickener or surfactant allows for better coating and absorption when using aqueous solvents. Aside from these adaptations for device extracts, the test methods are the same as those for chemicals.

The basic methodology is as follows. Young (8–12 weeks old), nulliparous, non-pregnant female mice of CBA/Ca or CBA/J strain mice are used. A minimum of four mice per group are used for chemicals. However, for medical device testing, since only one dose level is typically used, five mice per group are recommended. A 25 μ L portion of test extract or control solution is painted onto the dorsal surface of both ears of the mouse. This application is repeated daily for three consecutive days. At 72 h following the last application, animals are injected intravenously with the radioisotope, 3 H-methyl thymidine. The isotope will be incorporated into rapidly dividing cells. If the test substance is a sensitizer, lymphocyte proliferation will be increased in the lymph nodes that drain the ears and thus radioactivity levels will be higher. At 5 h following the isotope injection, animals are euthanized, lymph nodes are collected and radioactivity is measured. The radioactivity level in the test animals is divided by the background level of radioactivity in the negative control animals to determine a 'stimulation index' (SI). If the SI is ≥ 3.0 , the substance is considered a potential sensitizer. It is recommended to use a weak sensitizer (hexyl cinnamic aldehyde, mercaptobenzothiazole, benzocaine) as a concurrent positive control. If a laboratory has a long history of consistent positive responses with their positive controls, they may elect to do periodic positive controls. The positive controls should be run at minimum of every six months or less.

7.5.7 Systemic toxicity (ISO 10993-11:2006)

Tests for systemic toxicity evaluate the generalized effects to organs and tissues following exposure of the medical device to an animal model. The term 'systemic' implies that leachable chemicals are absorbed in one location and distributed throughout the body via the lymphatic or the circulatory

system to cause an adverse effect at a distant location. With medical device testing, the exposure typically occurs through administration of extracts or solutions, or the implantation of a part of the device. Historically, systemic toxicity testing has been divided into durations, that is, acute, subacute, subchronic, and chronic. In general, the acute study duration is intended as an initial screen to assess for gross signs of toxicity. The acute toxicity study uses limited numbers of animals and endpoints evaluated are observational type parameters, that is, clinical signs and symptoms, and body weight. As studies increase in duration, the group sizes increase along with the parameters measured. For chemicals and pharmaceuticals, toxicity testing using rodent and non-rodent species is usually required. However, for medical devices, toxicity testing using rodents only is generally accepted. Table 7.9 lists the recommended group sizes for the various study durations.

Acute systemic toxicity

Acute systemic toxicity is defined as adverse effects occurring at any time after single, multiple or continuous exposures of a test sample within a 24 h period. Typically the observation period is several days to a week. The purpose of this initial toxicity screen is to determine if leachables or soluble chemicals are present that would cause some degree of toxicity.

The design for the acute toxicity tests used for medical devices can vary, but they are most often based on the USP systemic toxicity test or the OECD test guidance. In this method, device extracts are dosed by the intravenous and intraperitoneal routes. The USP method was originally designed for pharmaceutical containers, that is, drug vials. Since the vehicle or drug product vehicle for pharmaceuticals can vary, a set of four vehicles is used

Table 7.9 Recommended minimum group sizes (ISO 10993-11:2006)

| Study type | Rodent | Non-rodent |
|------------|--------------------------------|----------------------------|
| Acute | 5 | 3 |
| Subacute | 10 (5 per sex) ^a | 6 (3 per sex) ^a |
| Subchronic | 20 (10 per sex) ^a | 8 (4 per sex) ^a |
| Chronic | 40 (20 per sex) ^{b,c} | ^c |

^a Testing in a single sex is acceptable. When a device is intended for use in only one sex, testing should be done in that sex.

^b The recommendation refers to one dose-level group testing. Where additional exaggerated dose groups are included the recommended group size may be reduced to 10 per sex.

^c Expert statistical consultation for chronic study group size is recommended. The number of animals tested should be based on the minimum required to provide meaningful data. Enough animals must remain at the termination of the study to ensure proper statistical evaluation of the results.

in this method: (1) saline, (2) 5% alcohol in saline, (3) vegetable oil, and (4) polyethylene glycol (PEG). For ISO 10993 testing, saline and vegetable oil extracts are considered sufficient for this screening assay. In this test method, mice are used and dosed intravenously (saline-based materials) or intraperitoneally (non-saline solution). Mice are observed for clinical symptoms of toxicity such as lethargy, hyperactivity, convulsions, weight loss and death. Animals are observed for a minimum of three days. For extracts a relatively large-dose volume, 50 mL/kg of body weight is used. While this dose volume is large, it is tolerated by mice. Since the quantity of leachables is generally expected to be low, this high-dose volume improves the sensitivity of the assay. However, this high-dose volume can present issues when extracting devices that are soluble. When testing a soluble medical device, a physiologically compatible solution should be prepared. The arbitrary 50 mL/kg dose should not apply, but rather the dose should reflect a multiple of the clinical dose on a mg/kg basis. This exaggeration factor is frequently 10–100 times the clinical dose.

Subacute/subchronic toxicity

The testing guidelines in ISO 10993-1 groups both subacute and subchronic toxicity in the same general biological effect category. Subacute and subchronic differ in duration of exposure. Subacute systemic toxicity is defined as adverse effects occurring after multiple or continuous exposure between 24 h and 28 days. Subchronic systemic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test sample for up to 90 days or not exceeding 10% of the animal's lifespan. The rationale for selection of either a subacute or subchronic test should be based on the clinical duration of use for the medical device, the nature of exposure, and the overall testing strategy. The method of exposure in these tests is most often by injections of extracts (intravenous and/or intraperitoneal) and by implantation. Selection of a route of exposure is based on clinical use of the device. The injection of extracts is frequently used for devices that contact the body via fluids, that is, haemodialyzer and prolonged-use catheters (vascular and urinary). This method is also useful for evaluation of devices that have indirect body contact or are externally communicating and not intended for implantation. For implanted devices, usually a portion of the device is implanted subcutaneously, intramuscularly, or intraperitoneally to provide the exposure dose.

For extract injection methods, rodents (mice or rats) are injected daily with freshly prepared devices extracts. The injection volumes are typically 10–20 mL/kg body weight. *Since an extract is used, the relationship to the clinical dose or exposure is not defined, and as such, a safety factor cannot be assigned to the dose.* Animals are observed daily for signs/symptoms of

toxicity, body weights are collected weekly, and food consumption may be conducted weekly (option). At the end of the test period, 2–4 weeks, blood samples are collected for clinical pathology analysis, animals are euthanized and a necropsy is conducted. Organs are weighed for group comparison and processed for histopathology evaluation. While ISO 10993-11 does not dictate a specific testing protocol, this current standard provides guidance on suggested parameters that should be evaluated.

For subacute/subchronic studies where the device is implanted, rats are most often used for these studies and parts of the device are implanted subcutaneously. The subcutaneous tissue along each side of the back is used most often as it can more readily accommodate larger pieces of a device. Selection of a ‘dose’ should be based on the clinical dose of the device. This is best determined on a weight basis. Using the device weight and patient weight (70 kg as a standard weight for adults), a clinical dose is calculated (mg or g of device/kg body weight). To improve the sensitivity of the assay, a safety factor is assigned to the animal dose, 100 times if possible. The size of the device will dictate the safety factor that is possible. For small devices, such as a coronary artery stent, this is quite feasible. However, for large devices, such as the polymeric portion of an artificial joint, a 10 times safety factor may represent a challenge due to the amount of material. As a general guideline, samples for subcutaneous implantation should be no more than approximately 1.5–2 cm across and 2–3 mm thick with rounded edges. To achieve a given dose, multiple specimens, up to three per side, can be placed in each animal. The duration of these studies range from four weeks to three months. The parameters evaluated throughout the course of the study and at the end are the same as with the extract injection method. An advantage of the implant design is that the implanted specimens provide tissues for the evaluation of local effects following implantation. So this design can address both subchronic toxicity and implantation (ISO 10993-6) requirements.

Chronic toxicity

Chronic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test sample for a major part of the life span. For rodents, this is usually considered to be six months in duration. The study design and endpoints evaluated are similar to the subchronic toxicity. For this longer duration study, the numbers of animals per treatment group are larger to account for possible losses over the course of the study and to improve statistical power. Depending on the nature of the device/biomaterial, duration of exposure and the results in subacute/subchronic toxicity tests, chronic toxicity may be sufficiently addressed through a risk assessment.

7.6 Conclusion

In order to perform a risk assessment of any medical device, information needs to be gathered that provides insight in the possibility that the use of the medical device might result in harm for the patient. The aim should be to reduce the risk as much as possible. One way of doing this is the use of non- or low-toxic chemical components and (bio)materials. The ISO 10993 series for the biological evaluation of medical devices provides a means to decide on the potential toxicity of a biomaterial or a medical device. Only a limited number of the *in vitro* assays are able to indicate specific hazards (e.g. genotoxicity, irritation) whereas other *in vitro* assays can be used for screening and mechanistic studies to select less toxic components for the production of a medical device. Also, the *in vivo* assays have their limitations, as the various animal species used may react differently to medical devices from humans. However, the combination of both *in vitro* and *in vivo* testing can provide sufficient information to decide on the potential toxicity of a medical device, which is important for the safety evaluation and risk assessment.

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Practical approach to blood compatibility assessments: general considerations and standards

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Abstract: Medical devices and medical device materials must be designed to achieve specific criteria for safety and performance. The criteria themselves may be bound by physical, chemical, and material limitations, and by biochemical, molecular, and cellular processes within the body recognized to take place upon contact. In the latter case, responses can be favorable or unfavorable, partially controlled by learned approaches, for example drugs, or be uncontrollable by current experience and know-how. As such, determining what testing is required and which test results are acceptable and meaningful in the development of a new medical device can be complex. This chapter highlights some of the main considerations in the specific area of evaluation of blood-contacting medical devices and materials. It discusses the main factors that may impact the blood–device/material responses that can occur, and it provides examples of some of the most common observations and approaches to manage the response. As the international standard ISO 10993-4 has been and continues to be a major driving influence behind this aspect of the biological safety evaluation of materials and devices, the evolving direction of the standard is covered and controversies in testing are reviewed. Ultimately, appropriate care taken to evaluate and understand critical blood–device/material interactions in each unique application leads to predictive and safe use in humans.

Key words: ISO 10993-4, blood–material interactions, blood compatibility, blood-contacting medical device, biological evaluation of medical devices.

8.1 Introduction

The past century saw remarkable developments in the areas of hematology, materials science, and medical devices. Yet, as the century drew to a close and numerous intersections in these fields arose, an impression remains that more could have been accomplished in terms of understanding and defining what makes a medical device (or material) safe and acceptable upon contacting and/or residing within blood. Thus, now well into the start of this century, it is fitting that this textbook should cover the essential and practical aspects of blood compatibility assessments and the present understanding of important blood–material/device interactions.

As we now know from cell and molecular biology, homeostasis is significantly driven by cellular interactions with the extracellular environment. Normal cell and tissue function is thus accomplished through a dynamic reciprocity of complex physical, chemical, and molecular interactions acting in concert (Bissell and Aggeler, 1987). Blood, a complex tissue itself, has significant potential to become altered from its normal homeostatic state by interactions that occur upon contact with a (foreign) material, such as a medical device. The compatibility of such interactions, i.e., blood compatibility, is then defined by the extent to which these new interactions can be deleterious to the host or the device. This chapter explores the current understanding of these interactions, and discusses current practical testing requirements and considerations. This is contrasted with the complexity of blood-contacting device applications, the host of potential interactions given the complexity of blood, and the array of materials applied in today's medical devices.

8.2 Background: blood composition

Since red blood cells (RBCs) were first observed in the sixteenth century, blood has arguably been viewed as one of the most complex fluids known. Appreciating what it takes for a material or device to be blood compatible accordingly requires at least a basic understanding of the composition and function of blood itself.

8.2.1 General composition

Compositionally speaking, approximately 8% of a body's weight is made up of blood, and a typical whole body blood volume is roughly five liters. Blood itself is composed of approximately 55% fluid elements (plasma) and 45% formed elements (cells). Plasma itself is composed of approximately 91% water, 7% dissolved proteins, and 2% other solutes, for example, hormones, gases, nutrients, waste products, lipids, salts. The dissolved proteins primarily consist of albumin (55%), globulins (38%), and fibrinogen (7%), i.e., 'the big three'. Within the cellular fraction of blood are erythrocytes (RBCs; ~5 million/ μL , ~6 μm diameter), platelets (~300 000/ μL , ~2 μm diameter), and leukocytes (white blood cells [WBCs]; ~7000/ μL , ~10 μm diameter). Including or excluding the contribution of the fraction of the small acellular platelets, the cellular fraction of blood consists of nearly 93–99% RBCs, respectively (Kaushansky *et al.*, 2010).

8.2.2 Formed elements and their function

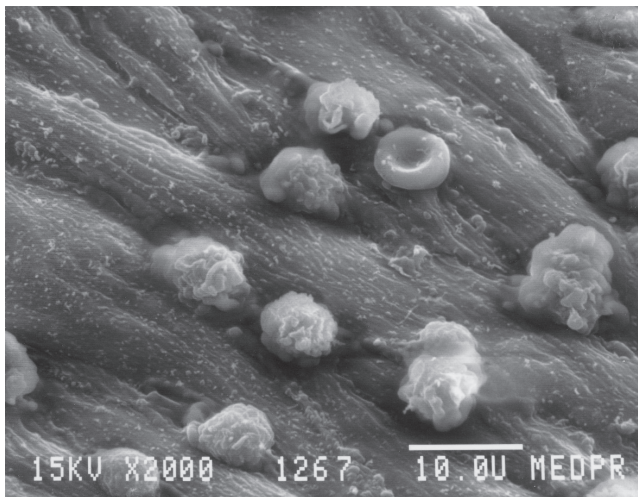
Blood performs essentially three major functions for the body: (1) transportation of critical elements through the body, for example, oxygen and carbon dioxide, food molecules (glucose, lipids, amino acids), ions (e.g., Na^+ , Ca^{2+} ,

HCO_3^-), wastes (e.g., urea), hormones, and heat, (2) defense of the body against infections and other foreign materials, and (3) maintaining hemostasis i.e., the complex balance between mechanisms that promote and prevent/mitigate coagulation and thrombosis. The fraction of blood cells dedicated to gas transportation speaks to the importance of this function. As is now well understood, oxygen dissolves poorly in fluids such as water and blood plasma, yet in the presence of the special protein hemoglobin that resides within RBCs oxygen is taken up almost 70 times more efficiently. Carbon dioxide, a waste product of cellular respiration, is also effectively handled by RBCs through binding at a site different from oxygen or being converted to bicarbonate and released to plasma. Other examples of the important transportation function of blood include the circulation (patrolling) of WBCs to guard against infection, the role of the plasma protein albumin to carry important hydrophobic substances such as lipids, vitamins, and steroid hormones, and the passive transport of waste material such as urea in plasma and RBCs to the kidneys.

Defense against infections and other foreign materials in the body is supported by essentially six different types of WBCs. Neutrophils, also commonly referred to as polymorphonuclear leukocytes (PMN) – describing their multi-lobed nuclei – are the most common type of WBC. They account for about 65% of all WBCs and act as the primary defenders (‘first responders’) against bacterial and fungal infections. They are seen to be present in all processes that involve inflammation. The much less common eosinophil accounts for about 4% of leukocytes and these cells primarily deal with parasitic infections. They are also the predominant inflammatory cell in allergic reactions. Basophils, on the other hand, make up only about 1% of WBCs. These cells are chiefly responsible for allergic and antigen responses via release of the chemical histamine, which causes vasodilation. Together, neutrophils, eosinophils, and basophils are referred to as granulocytes due to the appearance of small granules in their cytoplasm. These granules are actually small vessels of enzymes in membrane-bound ‘packets’ called lysosomes. Lysosomes contain potent enzymes used by the cells to digest bacteria and other foreign materials.

The leukocytes characterized by the absence of granules in their cytoplasm are lymphocytes, monocytes, and macrophages. Lymphocytes are the second most prominent WBC and they account for approximately 25% of the total WBC count. Lymphocytes consist of three subtypes: B cells, T cells, and natural killer cells. B cells make antibodies that bind to pathogens to enable their identification for destruction. Some can have long life spans and show ‘memory’ by retaining ability to produce specific antibodies. T cells have a number of subtypes that together act to coordinate and facilitate the immune response. They are also important in the defense against intracellular bacteria, viruses, and tumor cells. Natural killer cells received

their name because of their special capacity to recognize and kill cells of the body that display a molecular signal indicating infection by a virus, or a cancerous phenotype. Monocytes make up about 6% of WBCs and are long lived compared to other WBCs. They reside in blood and scavenge for bacteria, viruses, and other waste and foreign materials that need removal – using a process called phagocytosis. After phagocytosis, the monocyte can present certain components of the phagocytized material on its cell surface. Passing T cells then ‘learn’ about the chemical make-up of material/pathogen from the surface moieties, making further pathogen detection and destruction much easier. These cells also possess the unique capacity to reach beyond the blood stream through extravasation – the passage from the blood stream into damaged tissue through the vessel wall endothelium (Fig. 8.1). This movement is driven by chemical substances (chemotaxis), which can be triggered by a variety of stimuli such as damaged or dead cells, pathogens, foreign materials, and cytokines released by macrophages already present at the site. Once a monocyte has moved into the body tissues, it undergoes differentiation and becomes reclassified as a macrophage, which can remain stationary or continue to be mobile and further seek out and engulf target materials. Once in tissue the macrophage is vital to the regulation of immune responses and the development of inflammation. They are known to produce a wide array of powerful and toxic chemical substances, including reactive oxygen species, enzymes, complement proteins, and regulatory factors. If production of these potent molecules continues unchecked, not only are the undesirable materials destroyed, but viable cells and tissues, and medical device materials, around the



8.1 WBC extravasation through vascular endothelium.

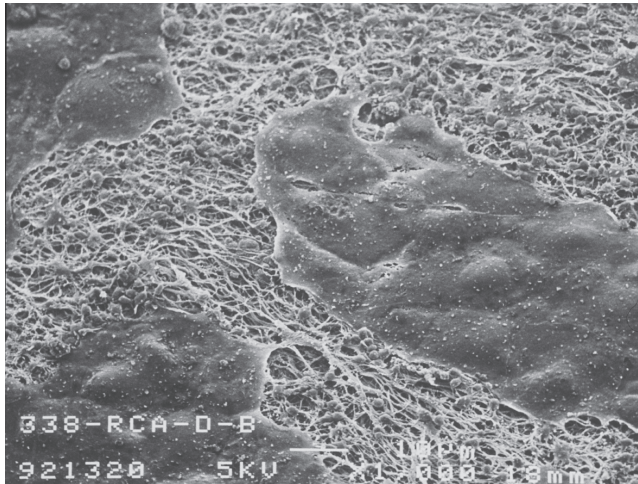
macrophages may also be impacted. The special role and function of the small anuclear cell fragment, the platelet, will be addressed in a following section, given its critical association with the coagulation cascade in the processes of hemostasis and thrombosis.

8.2.3 Plasma proteins, platelets and their functions

As mentioned, plasma proteins primarily consist of ‘the big three’ – albumin, globulins, and fibrinogen. Beyond the big three, and in much smaller concentration, are a number of key proteins that can play a major role in medical device performance. These key proteins, and the unique role of platelets, are discussed here.

The coagulation cascade and the platelet

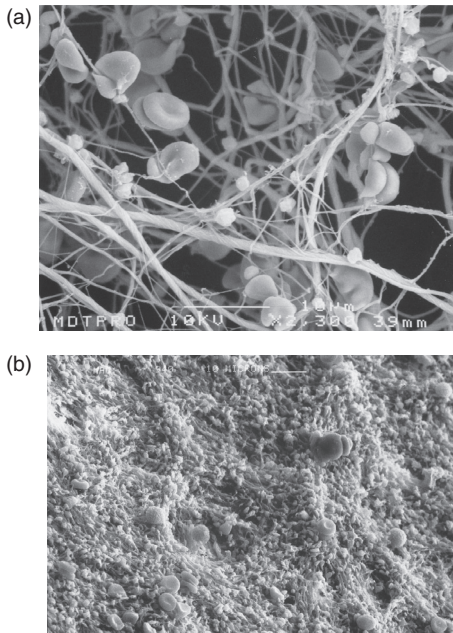
Along with providing defense mechanisms against foreign materials, the human body has highly evolved mechanisms to prevent blood loss – one of the blood’s most vital and important functions. This key function is also recognized to be a major factor in the performance of many blood-contacting devices. The natural reaction to injury to mitigate and prevent blood loss resides mainly with the small cellular fragment, the platelet, and the special collection of plasma proteins that make up the coagulation cascade. The platelet is often credited with providing the first line of defense by immediately binding to exposed and damaged tissue at the site of an injury. This reaction is assisted by a biorheological phenomenon referred to as platelet margination, i.e., the enhanced concentration of platelets that can occur in the near wall region of a blood vessel (Zhao and Shaqfeh, 2010). This initial response of *primary hemostasis* can also be assisted by other non-blood reactions such as vascular smooth muscle constriction (vasoconstriction) to slow blood flow, which can aid in providing opportunity for platelets to deposit and adhere. Platelet activation occurs almost immediately upon adhesion to the injury site. This starts with binding to exposed collagen fibers, where collagen-activated platelets then form pseudopods that stretch out to cover the injured surface (Fig. 8.2). During activation, they also go through a process of degranulation, in which potent additional activating agents are released from platelet granules (specialized secretory organelles), for example, ADP, platelet activating factor, platelet factor 4, fibrinogen and coagulation proteins V and VIII, promoting more precipitation of platelets. During activation, platelet membranes also expose receptors that bind circulating fibrinogen to their surfaces. In this process an aggregation of platelets and fibrinogen can rapidly build at the injured site to form a biological ‘patch’ or barrier to contain blood loss.



8.2 Scanning electron micrograph of injury to a coronary artery endothelium upon balloon expansion during coronary stent placement. In between patches of endothelial cells are regions of exposed subendothelial matrix proteins. Close inspection of the fibrous matrix shows numerous platelets in various forms attached to the prothrombogenic matrix proteins.

Secondary hemostasis occurs almost simultaneously with primary hemostasis. Here, coagulations proteins within the plasma respond in a complex cascade to form the critical protein thrombin, which catalyzes the formation of the protein fibrin. Fibrin forms into a mesh-like network that makes up the main protein element of the platelet patch. It strengthens it, particularly upon cross-linking by an endogenous protein (factor XIIIa), and often captures and snares RBCs and additional platelets to assist in further patch/barrier formation (Fig. 8.3). At this point the patch material is referred to as a thrombus. The appearance of thrombus is highly variable, as it can be clear to white (corresponding to thickness) when primarily composed of fibrin and platelets, or pink to red depending on the level of RBC entrapment. The latter is partly flow related, with higher RBC entrapment often occurring in lower flow environments, for example, venous blood flow.

The coagulation cascade leading to fibrin formation is composed of multiple precursor proteins that make up several distinct pathways. The *contact activation pathway*, which is triggered by elements that lie within the blood itself (thus its alternative name – the *intrinsic pathway*) is thought to start with three proteins: high-molecular-weight kininogen, prekallikrein, and factor XII. These proteins form a critical complex on exposed vessel wall collagen or on a blood-contacting device material. Factor XII then becomes



8.3 (a) Typical thrombus showing a rich concentration of fibrin protein strands decorated by platelets and entrapped RBCs. (b) Mural thrombus on a vascular graft wall showing a rich concentration of platelets (rather than fibrin) along with occasional red and white blood cells. Blood flow is left to right and Lines of Zahn (platelet rich wavelets) are apparent.

activated to XIIa, which initiates the coagulation cascade. A cascade of activation reactions involving other key coagulation factors ultimately leads to the critical Xa/Va (prothrombinase) complex, which catalyzes the conversion of prothrombin to thrombin (see [Plate I](#) in color section between pages 246 and 247). The *tissue factor pathway*, on the other hand, is triggered by damage to the vessel wall and to tissue outside of the blood vessel (thus its alternative name – the extrinsic pathway). Unlike the multiplex of proteins required to trigger the contact activation pathway, this pathway is triggered by a single protein that is present in subendothelial tissue – a protein appropriately called tissue factor (TF). In cascade nomenclature TF is also called factor III. It is expressed at the surface of tissue cells and its crucial function is to act as a cell surface receptor for factor VIIa, produced upon trauma. The TF–VIIa complex catalyzes the conversion of the inactive factor X into the active protease Xa. The catalysis of prothrombin (factor II) to active thrombin (IIa) by the Xa/Va complex and the subsequent conversion of fibrinogen (I) into fibrin (Ia) by thrombin is referred to as the *common pathway* (Plate I).

Maintaining hemostasis entails control of coagulation when it occurs and preventing or mitigating it when it is not necessary. A number of proteins carry out this function. Tissue factor pathway inhibitor (TFPI) is a polypeptide that can reversibly inhibit both factors Xa and thrombin. Complexed to factor Xa (Xa-TFPI) it also inhibits the VIIa-TF complex to prevent more Xa formation. The important protein antithrombin can also shut down the TF pathway through specificity to deactivate the active form of factor VII. Antithrombin, as its name implies, also has specificity to deactivate thrombin, yet it has equally important deactivation specificity for other key factors in the contact activation pathway that contribute to the amplification response (XIIa, XIa, Xa, and IXa). It is noteworthy that the rate of antithrombin's inhibitory activity is greatly enhanced through binding with the drug heparin, thus the main mechanism of action of this anticoagulant. The antithrombin deactivation rates against the various factors are greatly accelerated by heparin and vary from 10^2 - to 10^6 -fold increases. Protein C (also called factor XIV) is yet another important regulator of blood coagulation in that it is a potent deactivator of factors Va and VIIIa, which play such a crucial role in the formation of prothrombinase. Interestingly, the rate of protein C activation itself is recognized to be increased 1000-fold by a negative feedback loop driven by yet another combination of proteins – the thrombomodulin-thrombin complex.

Finally, once formed there needs to be an effective mechanism to remove thrombus when it is not needed. This is accomplished primarily through the enzyme plasmin that degrades many blood proteins yet most notably the fibrin within a thrombus. Like other pathways, this *fibrinolytic pathway* has a number of activating and inhibitory factors (see [Plate II](#) in color section between pages 246 and 247). See Kaushansky *et al.* (2010) for further details on the topics described in this section.

The complement system

One other series of plasma proteins of note with regard to blood-contacting medical devices, one designed to assist the cellular defense mechanisms previously mentioned, involves certain plasma proteins that 'complement' the ability of antibodies and WBCs to recognize and remove pathogens and other foreign materials. This system, aptly referred to as the complement system, consists of more than 30 unique plasma and membrane-bound proteins and involves three separate pathways. Like the non-specific (non-antibody/antigen) and specific (antibody/antigen) cell-based responses to foreign materials, the complement system proteins can act through either an intrinsic mechanism (the alternative pathway) or a mechanism mediated by specific recognition elements (the classical

and lectin pathways). Collectively, these pathways make up the underlying detection mechanism for protecting the host against foreign materials. Once triggered, complement proteins are cleaved into active fragments through a cascade reaction. The cascade can amplify proteins that bind to and tag the foreign material and/or act as unbound signals. The most recognized functions of these proteins are to cause sublytic cell activation or cell death. This is accomplished via forming ‘membrane attack complexes’ (MACs) to elicit an inflammatory response (particularly through C5a) via receptor-mediated activation. Adhesion and degranulation of granulocytes, monocytes, and mast cells (histamine release), and/or recognition and phagocytosis by WBCs of ‘tagged’ (complement-coated) materials are also triggered. Given the immediate and general response provided by the alternative pathway, which does not require a specific antibody to commence, and the fact that antibody formation against most common medical materials is rare, it is the alternative pathway response that is most responsible for complement activation that can take place in response to a medical material. The alternative pathway is shown in [Plate III](#) (see color section between pages 246 and 247). (See Johnson, 2004.)

8.3 Critical distinguishing factors presented by blood-contacting medical devices

In each blood-contacting application, key *device- and application-related* factors can influence the responses in blood itself and/or the response(s) of or upon a particular material or device component. Some of the most significant factors are: the material present and its surface chemistry and topology; the blood-contacting surface area; the duration of exposure; and the type of contact (direct or indirect, dynamic or passive).

8.3.1 The material(s)

Decisively, the material(s) used in the manufacture of most of today’s blood-contacting devices are chosen from a short list of highly tested and approved materials that primarily satisfy each application’s physical and material requirements (Table 8.1). This approach is driven by the fact that device-approval processes can be enormously less demanding, less costly, and less risk-prone when proven and approved materials are used. (Notably, the opposite applies when using new non-clinically-tested materials.) And arguably, when separated by certain basic surface properties, such as hydrophobicity and surface area, many of the conventional materials present remarkably similar responses in blood (barring some special surface-modified

Table 8.1 Commonly used medical device materials

| Type of material | Implant duration < 24 h, 24 h to < 30 days, > 30 days | Example applications |
|-------------------|--|---|
| Polymers | Polyethylene (PE), ultra-high molecular weight PE (UHMWPE), highly cross-linked polyethylene (HXPE), polyetheretherketone (PEEK), polypropylene (PP), polybutylene (PB), polyvinylchloride (PVC), polyvinylalcohol (PVA), polystyrene (PS), polycarbonate (PC), polyamides, polytetrafluoroethylene (PTFE), high-performance polyurethanes (PUs) and silicones e.g., PDMS, polyethylene glycol (PEG), polyglycolic acid (PGA), polylactic acid (PLA), polylactic-coglycolic copolymers (PLGA), polymethylmethacrylate (PMMA), cellulose and modified cellulose | Sutures, medical textiles, molded plastic components, tubings, e.g., pacing leads, cardiopulmonary bypass equipment, catheters and cannula, vascular grafts, heart-valve sewing rings, annuloplasty rings, bandages, condoms, breast implants, absorbable sutures, bone cement, spinal implants, contact lens, intraocular lens (IOL), hemodialysis |
| Metals | Stainless steel, e.g., 316L, cobalt alloys (Vitallium), titanium (pure) and titanium alloys, e.g., Ti-6Al-4V and medical-specific alloys, titanium-nickel alloy (Nitinol), titanium nitride, platinum, iridium-platinum, MP35N, gold, tantalum, palladium, silver (antimicrobial), magnesium and magnesium alloys | Surgical instruments, suture wires, needles, bone screws, mechanical heart valves, artificial joints, e.g., hip implants, stents, dental implants, pacing and neural stimulation electrodes |
| Ceramics | PC, alumina, alumina oxide, silicone carbide, hydroxyapatite, zirconia composites (ZTA, YTZP, PZT) | Orthopedic applications, mechanical heart valves |
| Biological tissue | Cadaveric tissues, and processed* allogeneic and xenogeneic (porcine, bovine) tissues, fibrin | Tendon and bone replacement, bioprosthetic heart valves, fibrin glues |

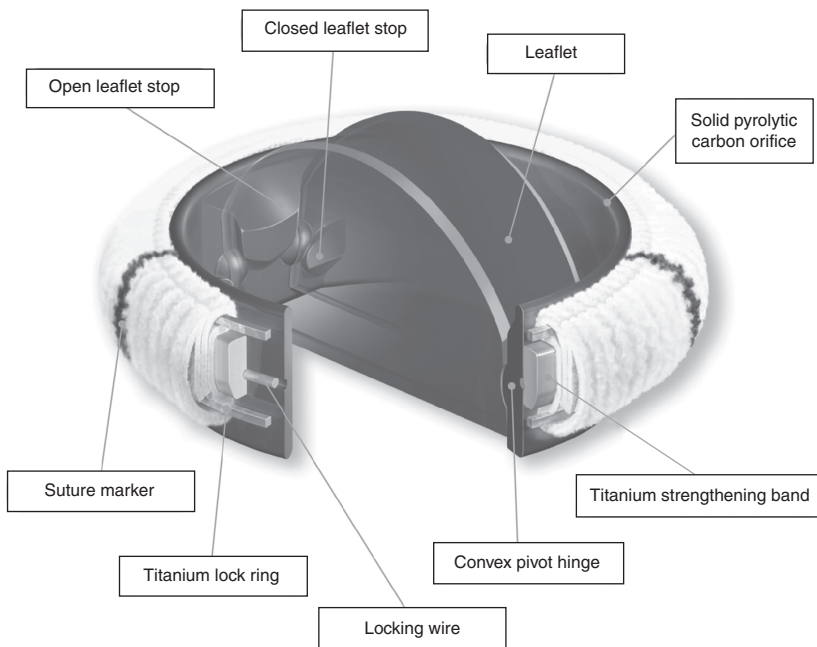
*Decellularization, freeze drying, cross-linking, sterilization, etc.

ZTA = zirconia toughened alumina, YTZP = yttria-stabilized polycrystalline tetragonal zirconia, PZT = lead-zirconate-titanate

materials). A case example is a pacemaker lead, the blood-contacting component of an implantable pacemaker. This permanent-contact device passes through the venous system to deliver electrical pacing to the right side of the heart. The blood-contacting portion of today's pacing leads is primarily

composed of one of two polymers – high-performance polyurethane or silicone. These two polymers were selected primarily because of their physical and biostability properties (Ebert *et al.*, 2011), and only secondarily because of their particular interactions with blood. Studies on the latter suggest that polyurethane leads may have slightly less platelet reactivity (in radiolabeled platelet studies) and potentially a degree less surface thrombus when used clinically (Palatianos *et al.*, 1994; Goto *et al.*, 1998; Martin *et al.*, 1999; Reinig *et al.*, 2007). Yet, neither material has been linked to a different or unacceptable level of risk or complications due to an adverse response in blood.

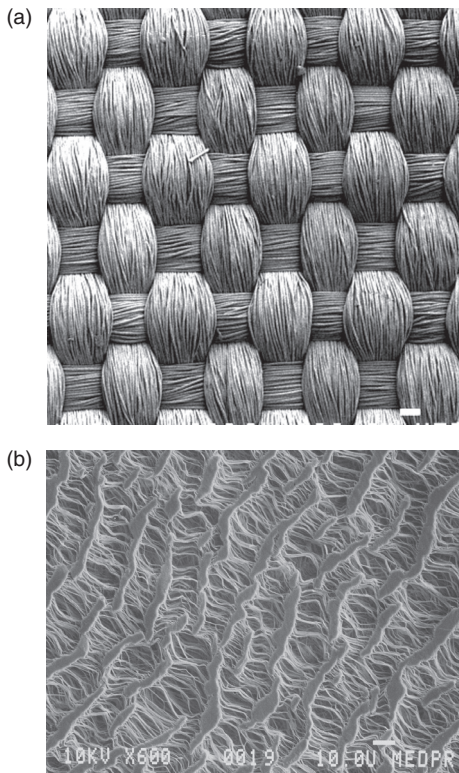
Materials that perform well by themselves in contact with blood may also be combined to form more complicated composite devices. For example, a single modern mechanical heart valve may be composed of pyrolytic carbon (PC), polyester (PET) fabric, various suture materials, and titanium. The valve housing and leaflets are made of PC, with the rest of the materials used in the sewing ring and for its attachment (Fig. 8.4). Each material used in this application was selected because of its physical properties and safe history of use in this application (and similar uses). There have been few if



8.4 A mechanical prosthetic heart valve. The valve itself can be composed of numerous blood compatible materials including PC for the housing and leaflets, and Dacron™ fabric in the sewing ring. Other materials such as a titanium components and marker band dyes may also be present.

any reported instances of negative interactions leading to unacceptable biocompatibility occurring from combinations of high-performance materials.

The interface between differing materials is often a point for scrutiny. Transitions that are not smooth may lead to unnecessary turbulence in blood, and gaps may offer opportunity for blood stasis. The strategy of minimizing their occurrence within reasonable manufacturability limits is the most common path taken, as risk of an associated adverse event is low and very difficult to ascertain (see Gross, 1996). Similarly, general changes in topography/surface geometry are recognized to be potential points of thrombus build-up due to flow perturbations, yet in many cases differences in performance may not be perceptible. For example, significant surface topology differences exist in the commonly used vascular graft materials Dacron™ and ePTFE (Fig. 8.5) yet their clinical performance is quite similar. In other applications, however, surface topology has been seen



8.5 Woven Dacron™ (a) and ePTFE (b) vascular graft surface topographies. The surfaces show remarkably different surface geometries but actual clinical performance is similar. Scale bar in panel (a) represents 50 μm .

to impact performance. Replacement of some of the early and relatively smooth blood-contacting surfaces in first generation total heart and ventricular assist devices by rougher surfaces was seen to be associated with improved blood-contacting responses (Rose *et al.*, 1994; Zapanta *et al.*, 2006).

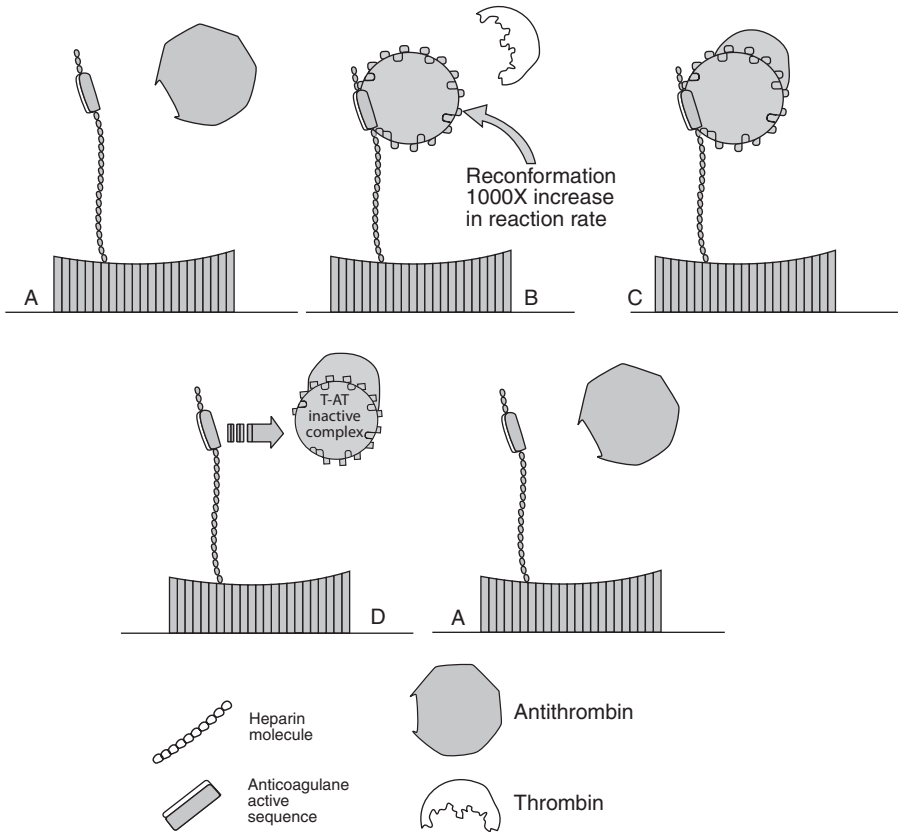
8.3.2 The material surface chemistry

The portion of a material that resides at its surface can present different chemical characteristics from the (bulk) material below the surface. This is because of the unique molecular bonding situation at the material surface, and to some extent the media present at the surface, for example, air, water, or blood.¹ It is this part of a material and its particular chemistry that is associated with the biological interactions that take place upon contact with blood. While many materials (surfaces) perform acceptably in blood-contacting applications, 'surface modification' or the altering of a material's surface to tailor a certain property or response is a new and evolving science in medical device applications. Surface modifications may be used, for example, to add simple lubricity to difficult manufacturing processes, to facilitate bonding of different materials, or to improve the blood-contacting response of certain materials. The bonding of the anticoagulant heparin to a polyurethane central venous catheter (CVC) is one example. This type of catheter is routinely placed into a venous environment for period of hours to days (up to 30) in the presence or absence of systemic anticoagulation. The polyurethane normally presents a highly hydrophobic surface, but with heparin surface modification, the blood-contacting surface becomes hydrophilic, bioactive, and non-thromboadherent. The theoretical biological mechanism of immobilized heparin is shown in Fig. 8.6. This reduction in thrombus may have important secondary effects, such as reduced opportunity for bacterial adhesion and infection in the thrombus on the catheter surface.

8.3.3 Material surface area and contact duration

The actual surface area (cm²) of blood contact presented by a device is recognized to play a significant role in the degree and type of blood response(s) to the device. What is more, surface area varies widely across applications. For example, a 3 cm long, 3 mm OD coronary stent, a 20 cm 7-French central venous catheter and a modern blood oxygenator present blood-contacting

¹ The presence of processing aids may also be a factor. Depending on its physical chemistry properties and interaction with the bulk polymer, processing aids may tend to bloom to the surface, reside in the bulk, or find an intermediate distribution, for example form islands on the surface.



8.6 The proposed mechanism of action of immobilized heparin on a surface. A: heparin attached to a solid material is oriented so that its active sequence can interact with blood elements; B: when heparin binds to antithrombin (AT), its tertiary structure changes resulting in a heparin-AT complex that has a much greater affinity for coagulation factors than AT alone; C: factor IIa (thrombin) in the blood flowing past the material binds to the heparin-AT complex and becomes inactivated; D: the thrombin-AT complex detaches from the heparin molecule and is eventually metabolized by the body. Similar to naturally occurring heparin sulfate on the vascular endothelium, immobilized heparin is not consumed by this cycle and remains available to attach to other AT molecules.

surface areas of roughly 1 cm², 15 cm², and 25 000 cm²! Thus, devices can present ten- to thousand-fold differences in blood-contacting surfaces, and this difference can influence the type and magnitude of interaction with important blood components. Similar orders of magnitude exist regarding contact duration. Some guide catheters, for example, only see direct blood contact for minutes. And in the examples above, a typical bypass surgery is 90 min, a

CVC implant can be for hours to days, and a coronary stent is a permanent implant and may be present for years to decades.

8.3.4 Direct versus indirect contact

Most of the blood-contacting medical device applications today involve direct contact with circulating blood, and to some degree, contact with blood in breached tissue. However, there are a number of device applications in which component materials exclusively contact blood through *indirect* exposure to intermediate carrier fluids. Examples of the latter are intravenous saline solutions, parenteral fluids, cardioplegia solutions, drugs from a delivery device, for example, a drug pump reservoir, and priming solutions and gases in a blood oxygenator. In each of these cases, the focus and concern is on the interaction of the delivery fluid with the materials, and on the impact of chemicals that may be potentially generated, extracted, and/or released and passed on to the patient's blood.

8.3.5 Contact dynamics

A final important device-related factor that can influence the response in blood is the contact dynamics. Devices such as mechanical heart valves, roller and centrifugal blood pumps, intra-aortic balloon and axial flow pumps, left ventricular assist devices, artificial hearts, and cardiac ablation catheters, etc., each impart an active physical–mechanical interaction with blood. In such devices, design features, such as shear forces and mechanical heat generation, must be carefully engineered to have minimal impact on blood, and the routine function of the device.

8.4 Responses in fluid blood in contact with medical devices

With the host of device applications and contact conditions set against the intricate composition and function of blood, the potential for numerous significant plasma- and cell-based responses would be presumably high. Fortunately, the number of recognized clinically significant reactions in blood to medical devices is not vast. The most recognized reactions that occur within blood itself are covered in this section.

8.4.1 Coagulation and thrombus formation

The natural mechanisms of platelet activation, blood coagulation, and thrombosis discussed earlier can occur to various degrees upon blood contact with

a medical device. Under normal homeostasis, blood in the body is maintained in a finely tuned balance of safe and background levels of coagulation and fibrinolysis. Contact with a device/device material initiates these mechanisms (Fig. 8.3 and Plates I and II) and they can remain local to the device or, left unchecked, they can potentially spread throughout the body vasculature. In the extreme case, small initial amounts of surface thrombus and free thrombus (thromboemboli) can grow and uncontrollably consume platelets and coagulation proteins. The thromboemboli themselves can disrupt normal blood flow in capillary and larger vasculature leading to tissue ischemia and organ failure. Almost simultaneously, natural negative feedback signals to the process of thrombosis (Plates I and II) can trigger the fibrinolytic system to respond to cause fibrin breakdown. Extreme responses here can cause the converse risk of excessive bleeding. While these various extreme responses rarely occur with medical devices, the response intensity is generally recognized to be proportional to the blood-contact surface area of the device and/or the extent of blood and tissue damage induced in the application. That is, the larger and more procoagulant a surface is on a device, and/or the larger the release of procoagulant factors from blood and tissue damage, the higher the potential for thrombosis. This includes a higher threat for overwhelming the body's capacity to control thrombosis through natural circulating anticoagulants, for example, antithrombin, proteins C and S, TFPI, and plasmin. Example applications that involve the highest device surface areas include cardiopulmonary bypass, extracorporeal membrane oxygenation (ECMO) for life support, and hemofiltration and hemodialysis. Section 8.3.5 provided examples of devices that may be associated with blood trauma and/or other tissue trauma. Importantly, a number of molecular biology tools exist to monitor the extent to which coagulation and thrombosis takes place (see Plate I). In conjunction with these tools that accurately measure proteins in the coagulation cascade, tools exist to assess extent of platelet activation, for example, via simple platelet counting, or via assaying for factors released from platelet granules, for example, BTG and PF4. More advanced tools such as fluorescence-activated cell sorting (FACS) analysis to assess secondary responses such as formation of platelet microparticles and platelet-leucocytes have also been applied (Gemmell *et al.*, 1995; Gorbet and Sefton, 2004; Chirinos *et al.*, 2005).

The importance of anticoagulant use for proper device function

Advances in the last century in the development of anticoagulant therapies have substantially mitigated the threat of excessive thrombosis and made a number of medical device technologies practicable. The anticoagulant warfarin, for example, has become one of the most commonly used oral anticoagulants. This drug has shown particular efficacy in the prevention of thrombotic

and thromboembolic events in patients with chronic atrial fibrillation, venous thromboembolism, coronary artery disease, and prosthetic heart valves² (Hirsh *et al.*, 2003). The glycosaminoglycan *heparin* is another potent anticoagulant. Once administered, it rapidly quenches existing coagulation cascade activity by stopping the formation new thrombin (hence more thrombus) through deactivating existing and nascent thrombin activity (see Section 8.2).^{3,4} In turn it allows the natural fibrinolytic mechanisms within blood to work to break down thrombus that has already formed. Heparin's ability to potentiate anti-thrombin activity against coagulation factors is now recognized to involve different segments (lengths) of the heparin molecule. This size-dependence on anti-factor activity prompted the development of new low-molecular-weight heparins (LMWH) to facilitate more subtle regulation of coagulation and improve heparin's therapeutic effectiveness (Weitz, 1997). Because of its short acting nature and reversibility with protamine sulfate, device applications such as cardiopulmonary bypass, ECMO for life support, and hemodialysis and hemofiltration have found the greatest use of heparin. The LMWH derivatives have found use in prophylaxis for a number of indications including acute coronary procedures, deep venous thrombosis (DVT), and prosthetic hip and knee replacement (where its use is less device-related but rather disease-, mobility-, and procedure-related, to protect against DVT) (Hirsh *et al.*, 2001).

The importance of antiplatelet drugs for proper device function

Along with anticoagulants to mitigate the potential for thrombosis are adjuvants that mitigate the level of platelet involvement in thrombosis. These antiplatelet drugs target the role of platelets as a first line of defense in primary hemostasis. One such drug, one of the most widely used medications in the world, is acetylsalicylic acid (aspirin). Aspirin exerts its antiplatelet effect by inhibiting the platelet production of the lipid thromboxane, which has several forms and

-
- 2 The mechanism of action of warfarin is to lower the amount of active vitamin K available as cofactor in activation of the clotting factors II, VII, IX, and X. The safety and effectiveness of this drug requires monitoring of coagulation activity using a prothrombin time (PT) coagulation test. This result is normalized to an international standard to give an 'international standardized ratio' or 'international normalized ratio' (INR). Monitoring of INR and dose adjustments of warfarin are frequently required to obtain a target INR value. Too high an INR may indicate a chance of bleeding; a too low INR may indicate inadequate anticoagulation and a chance for thrombosis. This task can be challenging as INRs can be influenced by many factors, for example, changes in diet, other medications, illness, liver disease, and other factors.
 - 3 A thrombus is the final product of blood coagulation *in vivo* and it occurs in a flowing blood environment via the aggregation of platelets and the activation of the coagulation cascade in blood plasma. Attached to a surface, for example, vessel wall, it is sometimes referred to as a mural thrombus; free floating thrombus is referred to as a thromboembolus. The term 'clot' is sometimes confused with the term thrombus. A blood clot technically refers to a precipitation of blood that occurs in an *ex vivo*, *in vitro*, or post-mortem static environment.
 - 4 Not to be confused with tissue plasminogen activator, heparin does not have the capacity to break down thrombus that has already formed.

functions. Thromboxane A₂, for example, is produced and released by activated platelets, and it can cause platelet activation, aggregation (via fibrinogen binding to expressed glycoprotein complex GPIIb/IIIa receptors), and vasoconstriction (Patrono, 1994). In medical device applications, aspirin is recommended after placement of coronary and carotid artery stents and after coronary and peripheral artery bypass grafting. The drug dipyridamole (Persantine™) works through the same mechanism. A growing number of antiplatelet drugs that work through different mechanisms have been found to be effective adjuvants in these same device therapies. Clopidogrel (Plavix™), for example, works by irreversibly inhibiting the platelet receptor called P2Y₁₂, an adenosine diphosphate (ADP) receptor involved in platelet activation and aggregation. The blockade of this receptor inhibits a critical conformation change in the GPIIb/IIIa glycoprotein that normally allows fibrinogen binding (Savi *et al.*, 2006).

8.4.2 Other responses despite anticoagulant and antiplatelet therapies

Despite the effectiveness of anticoagulation and antiplatelet drugs (and combination regimens), reducing thrombosis and platelet activity with drugs is not 100% effective. Post perfusion syndrome, a transient neurocognitive deficit sometimes observed after cardiopulmonary bypass, is speculated to be the result of tiny debris (cellular and protein based, and air bubble microemboli) that form during cardiopulmonary bypass and are passed on to the brain (Jensen *et al.*, 2006). The biological microemboli may be composed of any combination of potential materials, such as: fibrin, fibrin-entrapped RBCs, small aggregates of morphologically normal and pseudopodial (activated) platelets, partially and completely degranulated platelets, platelet membrane fragments and microparticles, RBC membrane fragments and microparticles, and platelets bound to monocytes and neutrophils i.e., platelet–WBC aggregates. Such debris may form from a combination of residual thrombotic processes and physical cell disruption (often generally assessed by examining RBC hemolysis – measured via standard plasma-free hemoglobin assay on plasma). The latter factors may play a role in the small percentage of heart-valve patients that experience valve thrombosis and transient ischemic attacks despite careful INR monitoring and Coumadin dosing. A small percentage of coronary stents also still fail due to early and late thrombosis despite standard antiplatelet therapy (Daemen *et al.*, 2007).

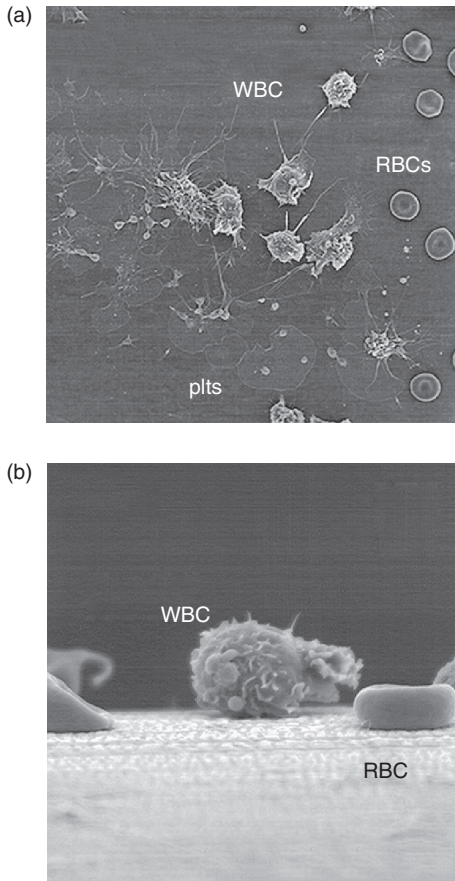
8.4.3 Complement activation

Another plasma-based reaction in blood that has drawn attention with blood-contacting devices involves activation of the complement system.

Complement activation has been implicated in certain adverse reactions during clinical extracorporeal therapies, in particular in hemodialysis (Craddock *et al.*, 1977; Chenoweth, 1984; Hakim *et al.*, 1984), and cardiopulmonary bypass applications (Chenoweth *et al.*, 1981; Velthuis, 1996; Fitch, 1999; Hsu, 2001). These therapies notably involve devices with high blood-contact surface areas and relatively short contact times. Here, activation starts with blood contact with the device material(s) and the deposition of numerous plasma proteins, including the critical complement proteins C3 and C3b. This contact leads to the alternative pathway formation of the crucial C3 and C5 convertase enzymes (see Plate III). The C5 convertase protein catalyzes C5 resulting in C5a and C5b generation. The C5a protein is a recognized effector of receptor-mediated neutrophil and monocyte activation, and the C5b fragment is the recognized initial complement component that leads to MAC formation – which binds to and activates and/or destroys bystander cells. WBCs can detect material surface-bound C3 and C4 fragments, which results in their subsequent surface adhesion and activation. Neutrophil and monocyte activation, MAC formation and activity, and WBC adhesion and activation (Fig. 8.7) on materials are the hallmark of the pathophysiology seen clinically in high surface area device applications. Importantly, Johnson *et al.* (1996) have shown the critical role of the fragment C5a in mediating many of these adverse reactions. Here, simulating exposure conditions during hemodialysis, purified C5a was infused into sheep and shown to present dose-dependent responses identical to those seen under actual dialysis. Continued work in this field has shown that materials whose surfaces are highly nucleophilic (hydroxyl- and amine-containing) present the highest complement activating potential, and that various surface modifications that reduce this type of surface chemistry greatly abrogate the classic clinical sequelae. This work is supported by other investigators studying the relationship of complement activation by medical device materials and the biological response (Johnson, 2004). As in assessing coagulation and thrombosis, a number of molecular biology tools exist to monitor the extent of complement pathway activation taking place (see [Plate III](#)). In conjunction with bioanalytical tools that accurately measure plasma complement proteins, methods exist to assess related responses such as WBC adhesion (using scanning electron microscopy) and WBC activation, for example, using bioassays for PMN elastase release.

8.5 Responses by materials, or upon their surfaces, in contact with blood

In parallel or sequential to hematological reactions that occur in fluid blood, blood contact with a device also results in reactions by the materials



8.7 Neutrophil and monocyte activation, MAC formation and activity, and WBC adhesion and activation on materials are the hallmarks of the pathophysiology seen clinically in high surface area device applications. Images are of WBCs attached to the porous hollow fiber component of a blood oxygenator: (a) low magnification; (b) high magnification. Occasional discoid RBCs are seen, along with flattened and spreading platelets (plts).

themselves and/or formation and accumulation of biological matter on the device surface.

8.5.1 Water movement

The impact and movement of water on blood-contacting materials is a common factor for consideration, given that plasma is comprised of greater than 90% water (or more correctly – 37°C, 0.9% saline solution). In this

environment metals can corrode and polymer materials can absorb water over time. Both of these factors, for example, were contributors in the phenomenon of metal-ion-induced oxidation (MIO) discovered in the 1980s. The metal ion oxidation was identified after observation of brittle cracking in pacing lead insulation made of a soft-grade of polyether polyurethane. The cracks started from the non-blood contact surface, i.e., at the lead's luminal side containing the metal conductor wires. It was here that cracking of the polyurethane developed following exposure to small levels of metal ions. The metal ions were derived from the corrosion reaction of the MP35N wire in contact with absorbed water. MP35N has a very slow corrosion reaction and is not usually detected in commodity applications. Despite extensive prior preclinical and clinical tests, however, this rare reaction was ultimately linked to insulation failures in pacing leads (Stokes *et al.*, 1989, 1995; Ebert *et al.*, 2011).

8.5.2 Degradation

Discovery and elucidation of the MIO failure mechanism on polyurethane pacing leads indicated the need for device manufacturers to consider and characterize other potential sources and mechanisms of material degradation. Towards this end, environmental stress cracking, or ESC, was discovered as a form of ductile cracking that can appear on a pacing lead external surface, i.e., where direct blood/tissue contact occurs and where residual stress may reside. This failure mechanism was found to occur only *in vivo*, as it was linked to chemical degradation reactions of specific polyether segments from oxidative agents produced by inflammatory cells. As mentioned earlier, cells such as neutrophils and monocytes migrate to the implant surfaces, where monocytes may further differentiate into macrophages. Here, macrophages may merge to form even bigger cell structures called foreign body giant cells. Together these cells produce oxidative agents in attempt to degrade the foreign material. At contact points, specific polyether components on the outside surface of the lead insulation are observed to be oxidized by these agents. The resulting polymer chain degradation, in particular in areas of residual stress, results in ductile cracking in the insulation surface (Zhao *et al.*, 1991; Casas *et al.*, 1999; and Lyu *et al.*, 2009).

8.5.3 Build-up of biological material

The nature, tenacity, location, and course of development of biological matter that accumulates or forms on the surfaces of blood-contacting devices ultimately plays a key role in defining and characterizing its blood-/bio-compatibility – see Table 8.2.

Table 8.2 Types of biological materials that form and accumulate on device blood-contacting surfaces

| Biological material | Time to form | Composition | Thickness | Blood compatibility |
|--------------------------------|------------------|--|------------------------|---|
| Protein layer | Seconds | Monolayer or multilayer of plasma proteins | 1–10s of nms | Low/variable |
| Acute thrombus | Minutes to hours | Fibrin, platelets, entrapped RBCs | 10s–100s of μ ms | Low/variable |
| Pseudointima* | Days to months | Cross-linked fibrin, collagen, other cellular elements | 10s–100s of μ ms | Med./variable |
| Anastomotic hyperplasia | Months to years | Collagen, blood vessels, proliferating FBs and SMCs | 100s of μ ms | High/variable (often due to flow perturbation and/or restriction) |
| Fibrotic tissue [†] | Month to years | Fibrous/collagenous semi-vascular tissue | 100s–1000s of μ ms | Med./variable |
| Reoccurring chronic thrombosis | Weeks/ongoing | Same as acute thrombus | 10s–100s of μ ms | Med./variable |
| Endothelium/neointima | Months to years | Endothelial cells on a stable matrix | 10s–100s of μ ms | High |

Notes: The extent and amount of biological materials that form depends on a host of factors, most notably time. The blood compatibility of the material can range from low to high, and may be variable at times due to factors such as developing flow perturbations caused by the materials.

* This name is used, for example, when the device in question is a vascular graft.

[†] This tissue can have various names depending on the device application. As mentioned, for vascular grafts it is called a pseudointima; for heart valves it is called pannus outgrowth; for pacing leads it is called chronic encapsulation tissue. FB = fibroblast; SMC = smooth muscle cell.

Proteins, thrombus, and thromboemboli

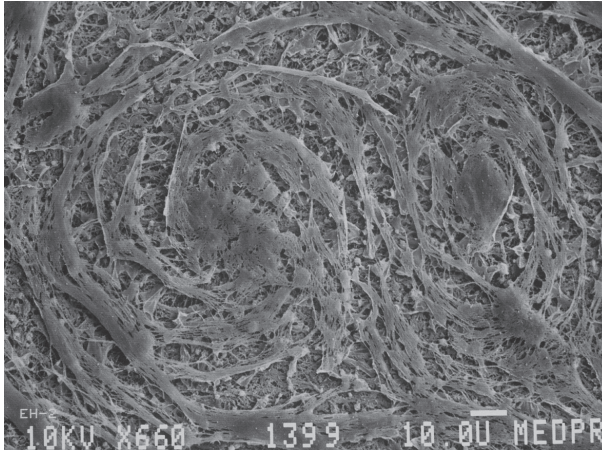
Immediately upon exposure to blood, plasma proteins become attached to the surface in a manner now described as the Vroman effect. These proteins have the potential to be passivating to a surface if they are relatively inert or, as described earlier, some have the capacity to act as initiators/activators of processes, for example, platelet deposition, blood coagulation, and complement pathway activation. Once platelet deposition and coagulation processes

have initiated to form a mass on the surface, such material is often referred to as acute thrombus, which takes on the form of a fibrin/platelet/RBC-rich mass (Fig. 8.3). The health risk associated with the presence of acute thrombus, i.e., its biocompatibility,⁵ very much depends on the application. For example, acute thrombus on heart-valve sewing rings and vascular grafts is recognized to be an important element of device healing. Here, early thrombus can promote healing and help minimize undesirable events – such as perivalvular leakage and graft seroma. The same acute thrombus on the exterior of a venous or arterial catheter (see [Plate IV](#) in color section between pages 246 and 247) about to be removed, however, may be potentially threatening as it can embolize and cause a potential downstream infarction (depending on embolism size, venous vs arterial location, etc.). Given the body's innate capacity to maintain hemostasis, however, in short time such thrombus is often biologically erased by host fibrinolytic mechanisms. Thus, its transient presence on some devices (such as pacing leads) is rarely a concern or source of complication. Alternatively, when device function calls for maintaining blood flow through a lumen, for example on a vascular graft wall or in an arterial bypass cannula lumen (Plate IV), continued active and developing thrombus can threaten device function and patency.

Pseudointima formation

A pseudointima is generally considered to be the biological material that lines the blood-contacting surface of a permanent vascular implant device. It is composed primarily of collagen, cross-linked fibrin, and organized or organizing thrombus, and it lacks a stable (natural) endothelial cell lining. For example, a pseudointima on a vascular graft wall (Fig. 8.8) or on the surfaces within a left ventricular assist device is thought to be reasonably inert and blood compatible (often with the help of an antiplatelet and anticoagulant drug regimen, and particularly with large diameter grafts). This same material can be problematic if it detaches and embolizes, or sheds small emboli. A case in point here involves artificial heart and ventricular assist devices where relatively smooth surfaces were eventually replaced with textured surfaces such as flocked polyurethane and sintered titanium (Rose *et al.*, 1994; Zapanta *et al.*, 2006). These device surfaces appear to promote more rapid and stable development and attachment of a pseudointima, and reduce risk of embolic problems (Szycher, 1986).

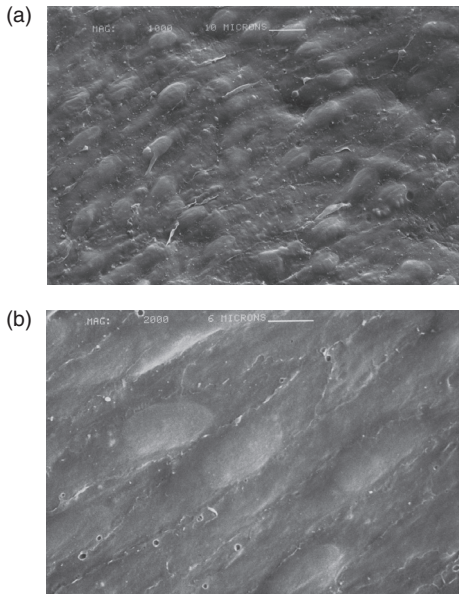
⁵ A well accepted definition of biocompatibility was stated by D.F. Williams in 1987: 'biocompatibility is the ability of a material to perform with an appropriate host response in a specific application' (Williams, 1987). This definition is based on the performance of the device. It does not rule out the existence of certain host responses; rather, it proposes accepting them as natural and tolerable (and inevitable) reactions as long as device performance and patient safety and quality of life are maintained.



8.8 Scanning electron micrograph of a pseudointima on the blood-contacting surface of a woven Dacron vascular graft. See Fig. 8.5 for an image of the same device surface taken before implantation.

Neointima formation and the importance of an endothelial cell lining

For many blood-contacting devices that are permanently placed in the vasculature, for example, vascular grafts, heart valves, coronary stents, annuloplasty rings, vascular patches, septal defect closure devices, etc., the ultimate surface would be one that resembles the healthy and stable vascular endothelium seen throughout the body (Fig. 8.9) seen throughout the body. It is this cellular layer that is recognized to be the most blood compatible surface known. Its rapid and full coverage is believed to mask a material's thrombogenic properties and minimize the potential for adverse interactions (Fig. 8.10). When the underlying cells and matrix of such a layer grow and thicken uncontrollably, it is often referred to as neointimal hyperplasia (anastomotic hyperplasia at the suture line of vascular grafts). Such phenomena can be very deleterious to device function. The capacity of humans to develop a stable and complete neointima on an artificial surface is unfortunately limited, in particular relative to a device's surface area and patient disease and health status (Wesolowski *et al.*, 1964; Berger *et al.*, 1972; Rafii *et al.*, 1995). For this reason, techniques that apply or attempt to entice formation of an endothelium on device surfaces have received significant attention. Some of the resulting techniques have demonstrated effectiveness and shown the concept to be a valid approach to improving device performance and material hemocompatibility (Laube *et al.*, 2000; Meinhart *et al.*, 2001). The methods required to harvest and cultivate these cells on device surfaces are not trivial, and an assortment of factors have limited large-scale use of the technology.

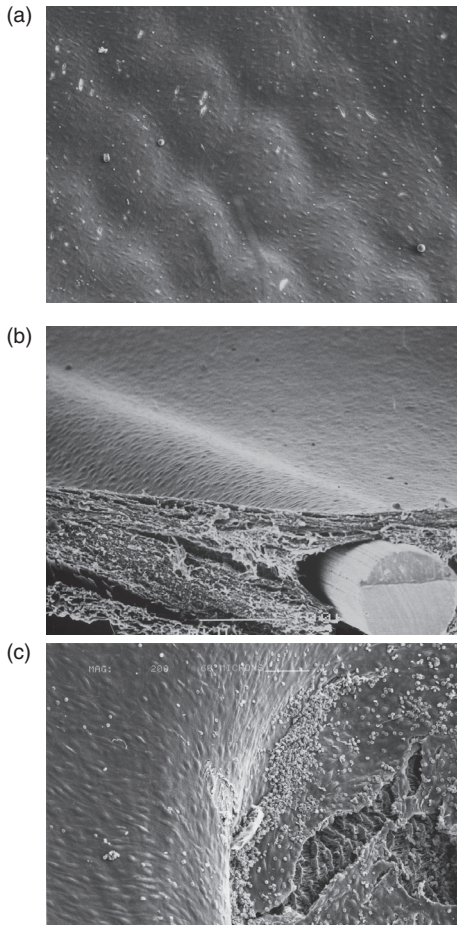


8.9 Confluent endothelial cells line the blood-contacting surface of the entire vasculature. (a) Arterial endothelial cells at low magnification; (b) same as panel (a) at higher magnification. Endothelial morphology can vary; ~20–25 μm long by ~5–10 μm wide spindle-shape morphology is common in arterial endothelium. Note that the typical ‘bumps’ seen on the surface reflect the underlying nucleus of each endothelial cell.

Encapsulating tissue formation

For long-term implant devices placed in the venous vasculature and into the right side of the heart, for example cardiac pacing leads, the most significant biological material found on portions of these devices is referred to as fibrotic encapsulating tissue (see [Plate V](#) in color section between pages 246 and 247). The initial events leading to its formation are thrombosis and endothelial injury, as proposed by Stokes and others (Huang and Baba, 1972; Stokes *et al.*, 1995; Cardinas *et al.*, 1999). Here, the presence of the device in the flowing blood presents a perturbing element to blood hemostasis, consistent with the theory of thrombosis that goes back to the time of Virchow.⁶ Recirculation or stagnation of blood around the lead body will cause thrombosis. Where the lead touches the endothelial cells lining the venous and cardiac walls, endothelial and potential subendothelial damage may occur. A consequence of this injury is thrombosis, primarily driven by the TF pathway. After time some device-associated thrombus undergoes

⁶ This theory states that hemostasis requires a proper balance of the blood’s coagulation state, flow, and the surface present (vessel wall, injury, foreign material).



8.10 A neointima on a vascular device consists of a stable healthy endothelial cell lining over an underlying stable subendothelial matrix. (a) Neointima over a woven Dacron vascular graft in a canine femoral artery model; (b) neointima over a Tantalum coronary stent strut in a porcine coronary artery model; (c) wave of neointimal hyperplasia (from the left) on a vascular graft in a canine model. Artifactual crack in neointima reveals the graft to be ePTFE (see Fig. 8.5).

fibrinolysis, yet other portions (particularly those involving tissue contact) undergo a reorganization process into a permanent (encapsulating) scar tissue. This tissue may present a pseudointimal (inert/acellular) or neointimal (endothelium-lined) surface morphology. In areas where there is continued blood recirculation or stagnation, and potential ongoing reinjury (e.g., due to dynamic motion), further thrombosis and thrombus organization is favored over removal by fibrinolysis. More thrombus may therefore

continue to form in association with the initial capsule. Because such tissue may be vascularized, new thrombus may continue to form and organize into more encapsulation tissue, allowing the tissue to slowly grow longer and/or thicker with time. One consequence of the latter is that as organized tissue grows thicker and denser, capillaries within it may atrophy. In time, the oxygen tension within the encapsulating tissue decreases, potentially triggering resident fibroblasts to differentiate into chondrocytes. A transformation of the tissue into cartilaginous morphology (called cartilaginous metaplasia) may follow. In the extreme case, resident cells within the capsule die, leading to a mineralization/hardening of the encapsulating tissue. Investigations in the field involving both animals and human explant tissues show that this process results in cases of early- and late-forming encapsulation tissue, and that encapsulation tissue formation, composition, and thickness are highly variable. For the most part this tissue is harmless to device performance but it can complicate device removal.

8.6 Assessing hemocompatibility according to international standards

With a significant proportion of the medical devices used today presenting a blood-contacting surface, and such devices spanning a variety of limited, prolonged, and permanent-contact applications, standardized methods for evaluating interactions with blood are necessary. The main international standard used to establish overall preclinical medical device safety comes from the International Organization for Standardization (ISO), and is entitled ISO 10993 Biological Evaluation of Medical Devices. Given the nature of the blood interface and its prevalence in medical device applications, this area of testing is treated as a separate section within the standard, entitled ISO 10993-4 Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood (ISO 10993-4, 2002/2006).

8.6.1 ISO 10993-4 Biological evaluation of medical devices: selection of tests for interaction with blood

Briefly, the ISO 10993-4 standard begins by offering common definitions and terminology, and categorizing the types of blood (body) contact into three general categories: non-contact devices (e.g., clamp that support a blood oxygenator on its exterior), external communicating devices (direct contact, for example, CVCs, and indirect contact, for example, drug pumps), and implant devices (e.g., mechanical heart valves). The guidance includes a process diagram to aid in deciding when actual new testing is necessary, as opposed to

when historical data and/or a supporting risk assessment may be appropriate. The potential types of significant blood interactions are classified into five categories, which are: thrombosis, coagulation, platelets, hematology, and the complement system. Tables provide examples of the categories worth considering⁷ in each application (separated into common externally-communicating devices and familiar implant devices – see Fig. 8.11). As a horizontal

| Device examples | Test category | | | | |
|--|----------------|-------------|-----------|----------------|-------------------|
| | Thrombosis | Coagulation | Platelets | Hematology | Complement system |
| Example external communicating devices | | | | | |
| Catheters in place for less than 24 h | x ^a | | | x ^b | |
| Catheters in place for more than 24 h | x ^a | | | x ^b | x |
| Blood storage and administration equipment, blood collection devices, extension sets | | x | x | x ^b | x ^c |
| Extracorporeal oxygenator system Hemodialysis/haemofiltration equipment Percutaneous circulatory support devices | x | x | x | x | x |
| Example implant devices | | | | | |
| Pacemaker leads | x ^a | | | x ^b | |
| Prosthetic (synthetic) vascular grafts and patches, including arteriovenous shunts | x ^a | | | x ^b | x |
| Stents | x ^a | | | x ^b | |
| Tissue heart valves | x ^a | | | x ^b | |
| ^a As stated in 3.7, thrombosis is an <i>in-vivo</i> phenomenon. It is recognized that coagulation and platelet responses are involved in this process. Therefore, it is up to the manufacturer, if specific testing in the coagulation and platelet test categories are appropriate for their device. ^b Hemolysis testing only. ^c Mandatory test methods are cited in Clause 2. Optional test methods are given in the Bibliography of ISO 10993-4. | | | | | |

8.11 Examples of circulating blood-contacting devices or device components and the categories of appropriate testing, as presented in ISO 10993-4.

⁷ These tables, and in particular the 'Xs' indicating areas for test consideration, have been a constant source of confusion to some users. The Xs have often been misinterpreted as indicating required testing. The intention of these Xs has always been that, based on the specific application, the user should consider the need and importance for testing in these areas. Then, when deemed important to establish safety, new testing would be conducted in each valid X. However, when deemed unnecessary, wasteful, or redundant, a rationale would be provided to explain the scientific basis for not conducting certain testing.

standard, this guidance document does not offer specific methodologies but emphasizes important common sense considerations and strategies for testing. Table 8.3 provides a list of the main considerations. Annex A in the standard offers some general considerations for preclinical evaluation on certain cardiovascular devices, and Annexes B and C offer information on the scientific basis of some of the specific tests and assays (C devoted to the subject of hemolysis testing).

8.6.2 Main tests

Unlike companion chapters in ISO 10993 that describe well-established *in vitro* test methods for genotoxicity, carcinogenicity and reproductive toxicity (ISO 10993-3, 2009), and *in vitro* cytotoxicity (ISO 10993-5, 2009), Part 4 has been slow to offer similar detailed and standardized methods for assessing device/material hemocompatibility. The main tests it currently advocates are the American Society for Testing and Materials (ASTM) hemolysis test,⁸ and testing for complement activation. The hemolysis assay has the form of a cytotoxicity test where human or animal RBCs are used rather than the mouse L929 fibroblastic cells applied under Part 5 cytotoxicity testing. This assay tests for the presence of elements that give rise to RBC toxicity and release of hemoglobin. The test itself is normally run on either an extract solution of the test device/material(s), i.e., the indirect method, or it is run using direct blood contact with the device/material(s), i.e., the direct method. A common over/misapplication of this test comes from uncertainty when to run one or both methods. For example, the direct contact method should not be necessary in a drug pump reservoir evaluation since the reservoir itself never experiences direct blood or tissue contact. Yet, sometimes it is mistakenly performed despite the fact that only potential extracts from the reservoir may become (indirectly) introduced into the body (via the drug carrier fluid). Complement testing, on the other hand, has universally relied on commercially available enzyme-linked immunosorbent assays (ELISA) designed to measure *in vivo*-formed complement proteins. Here, method adaptation has been necessary as complement formation occurs *in vitro* in a test tube where test materials are exposed to blood. Testing includes comparison to various known positive and negative control materials. To gauge the capacity of the material to activate the classical and alternative pathway, respectively, complement testing has evolved to call for measurement of the C3a and SC5b-9 fragments (see Fig. 8.2).

⁸ The ASTM hemolysis method (ASTM, 2009) is currently the most widely used assay to assess the hemolytic property of medical devices and materials. Other assays, such as the National Institutes of Health method (NIH, 1977), are also used, and some governments have adopted their own hemolysis assay to their scientific preferences — such as Japan (MHLW, 2003).

Table 8.3 Commonsense considerations when testing blood–material/medical device interactions (from ISO 10993-4)

| Section | Consideration |
|-------------|--|
| 6.1.1 | Vertical standards on specific devices take precedence over ISO 10993-4 |
| 6.1.2 | Tests must use models that simulate actual in-use application conditions, e.g., geometry, flow, contact duration, temperature, sterility, etc. |
| 6.1.3 | Appropriate traceable controls must be used, e.g., predicate device, positive and negative controls |
| 6.1.4 | Test actual complete (finished) devices (or components) only |
| 6.1.5 | Testing in poorly simulated use conditions will not be highly predictive of performance in clinical applications |
| 6.1.6 | Devices with <i>ex vivo</i> application, and devices with <i>in vivo</i> application, should be testing in appropriate <i>ex vivo</i> and <i>in vivo</i> models, respectively |
| 6.1.7 | <i>In vitro</i> tests may not be accurate predictors of blood–device interactions in prolonged, repeated, or permanent contact |
| 6.1.8 | Identify (1) type of contact, (2) best in-use test conditions, (3) necessity of testing – see ISO 10993-4 Fig 1, (4) appropriate test categories and tests, and (5) existing information before testing |
| 6.1.9 | Disposable lab equipment used for collection of blood for <i>in vitro</i> tests shall be evaluated for impact/non-interference on the test being performed |
| 6.1.10 | Species of blood and other factors may limit predictability of tests |
| 6.1.11 | Human blood should be used when possible; species differences in blood reactivity should be considered in model/test selection |
| 6.1.12 | Anticoagulant used in <i>ex vivo/in-vivo</i> models should be consistent with the type and quantity used in the routine clinical device application |
| 6.1.13 | Modifications to clinically-accepted devices must be considered for impact on blood–device interactions, e.g., changes in: design/geometry, surface chemistry, material, topography, porosity, etc. |
| 6.1.14 | Use appropriate replication, statistical design, and analysis methods |
| 6.2.1 | Consider tests, as appropriate, in these categories: thrombosis, coagulation, platelets, hematology, and complement activation |
| 6.2.2 | Non-contact devices do not require testing for blood–device interaction |
| 6.2.3–6.2.4 | Use Tables 3 and 4, to determine appropriate testing on external communicating and implant devices, respectively |
| 6.2.5 | Be cautious of test indications/limitations, e.g., immunoassay cross-reactivity, dilution factors, proper material characterization, appropriate in-use flow, etc. |
| 6.3.1 | <i>In vitro</i> tests should consider key factors such as: hematocrit, anticoagulant type and level, collection method, temperature, randomization, surface area-to-blood volume ratio, flow conditions, specimen handling/storage, etc. |
| 6.3.2 | <i>Ex-vivo</i> tests must be performed when the intended use of the device is <i>ex vivo</i> ; some <i>ex vivo</i> testing may be useful when intended application is <i>in vivo</i> |
| 6.3.3 | <i>In vivo</i> testing must be performed on devices intended for <i>in vivo</i> /implant applications. Protocols should include, as necessary, methods for appropriate assessment of blood/device interactions, e.g., analyses on (1) the device itself, (2) blood samples from the test subject, and (3) susceptible end organs |

8.6.3 Controversies

The hemolysis test has been a main stay assay used to screen materials for safe use in the body, and in particular for devices that come into contact with blood. With respect to the many important biological processes and functions present in blood discussed in this chapter, controversy around this simple test subsists as it gives poor/no insight into actual compatibility with these processes and functions. Even today, there continues to be a long-standing need for additional tests that address safety with the important mechanisms and functions residing in blood. Complement activation testing being one such example, was welcomed and quickly incorporated into the standard. Testing in this particular area, however, is also not without its own controversy. Inconsistencies in the use of anticoagulants, variation in source and preparation of blood for the test, and lack of supporting references regarding clinical significance (and predictivity) in various device applications remain as sources of continued debate and discussion. A linkage of complement activation to certain adverse events in devices used in hemodialysis treatment and cardiopulmonary bypass equipment initially drove the interest for a better understanding the of phenomena (Craddock *et al.*, 1977; Chenoweth *et al.*, 1981; Chenoweth, 1984; Hakim *et al.*, 1984; Velthuis, 1996; Fitch, 1999; Hsu, 2001). Importantly, this led to development of new materials that significantly reduce activation in these larger surface area/acute contact applications. Attention to complement activation in these device areas somehow also drove such testing to become a requirement in many regulatory agencies, with some requiring testing on all devices regardless of blood-contact surface area and implant duration. To date, no scientific papers or clinical reports of complement-related adverse events have been identified in other device applications, in particular with small-surface-area devices. Thus, appropriate references that link device-associated complement activation to adverse events in humans, along with a threshold device surface area of concern below which complement testing is not warranted, are lacking in the standard. It may be noteworthy that classic anaphylactic reactions have occurred in association with use of medical devices. Here, however, it has been generally attributed to an agent being delivered, rather than to a device or device material (e.g., Neidhart *et al.*, 1992; Laroche *et al.*, 1998; Bergamaschini *et al.*, 1995, 1996). Some false associations to the devices involved may have arisen from such reports.

In defense of the lack of standardized tests and methods in this area, there exists an overwhelming host of unique blood-contacting parameters and variables associated with each device application. As suggested in the many considerations described in Part 4 (Table 8.3), models must be designed to mimic the conditions expected in clinical use, for example, precisely controlled temperature, blood flow, test material surface area:blood ratio (cm²/mL blood), anticoagulant conditions, blood source, and contact duration. Applying these

very considerations, a number of *in vitro* models applicable to limited or specific devices have been developed (e.g., Münch *et al.*, 2000; Zimmermann *et al.*, 2007; Sukavaneshvar, 2008; Sinn *et al.*, 2011). Unfortunately, lack of validation and questions on relevance to *in vivo* responses have made such testing (and use of contemporary cellular and molecular analysis tools) more common in special characterization and feasibility studies. A small exception here is the *in vitro* use of radiolabeled platelets for platelet stability studies on storage containers for transfusions (Holme *et al.*, 1993; BEST Collaborative, 2006).

8.6.4 How standards can help to avoid controversies

One of the well-known advantages of having international standardized test methods is that they allow uniform evaluations to be conducted across different laboratories and geographies. This practice allows valid comparisons of results. Bringing together subject matter experts from across the globe, and the consensus process itself, also enables agreement on methods and helps to ensure that best methods are applied. When standards and consensus on methods do not exist, groups are forced to adopt their own preferred methods and controversies and discord may arise on methods applied. One case in point is the lack of a standard method to assess device/material thrombogenicity. One such method that has surfaced is the *in vivo* non-anticoagulated venous implant (NAVI) model. This test involves inserting a catheter-shaped device, or a device material made into a catheter shape, into the vein of a large animal. In the absence anticoagulation, it is allowed to incubate *in situ* for a period 1–4 h, then removed and assessed for amount of apparent thrombus on the surface. Here, the canine femoral vein model is commonly used and a control material or predicate device is simultaneously included in the contra-lateral vein. The test is repeated in duplicate or triplicate per test material/device, with scoring of thrombus on the device done using a method such as that shown in Table 8.4. Results may

Table 8.4 Typical scoring system applied to devices or device materials assessed using the NAVI model

| Score | Description of implant | Result |
|-------|--|--------------|
| 0 | Thrombus non-existent or minimal; if present, appears to be associated with implant venotomy site. | Pass |
| 1 | Thrombus minimal, observed to be covering 1–25% of material length. | Pass |
| 2 | Thrombus moderate, observed to be covering 26–50% of material length. | Intermediate |
| 3 | Thrombus severe, observed to be covering 51–75% of material length. | Fail |
| 4 | Thrombus extensive, covers 76–100% of material length. | Fail |

be supplemented with gravimetric analysis of thrombus and/or observations on vessel patency. [Plate VI](#) (see color section between pages 246 and 247) shows some representative images of test and control surfaces using the NAVI model. Where controversy arises is over the highly variable results that occur between: (1) test facilities, (2) test evaluators (3) replicates on the same material, and (4) in scores (often failing) obtained on approved and extensively field-tested clinical devices and materials. Many if not all of the latter materials have clinical histories that demonstrate that adverse events related to thrombosis are extremely rare, and often are not linked to the device or material but rather to other non-device factors. The inset below gives a summary of the main controversies of the NAVI model.

NAVI model caveats

The NAVI model is not widely accepted throughout the world and is acknowledged or required by only a few regulatory bodies. The controversy and caveats with the methodology are attributed to the following factors. Without extreme caution in methodology and expert interpretation, erroneous labeling of devices or materials as thrombogenic (or non-thrombogenic – see G below) may occur.

- A. **The implant position:** High-flow environments lead to low levels of surface-associated thrombus and vice versa. Therefore, slight differences in anatomical factors such as target vessel diameters and/or venous valve positions can have a significant impact on the amount of thrombus observed.
- B. **The implant technique:** Each test and control must be delicately and identically inserted into the target veins with each situated in identical position (preferably centrally-located, with no vessel wall or valve contact)
- C. **The extent of device–vessel wall contact:** This factor relates to vessel wall injury/endothelial denudation during the implantation and incubation periods. The sample itself can become an active bystander to rampant thrombosis initiated primarily through the tissue injury it inflicts on the vessel wall and resultant TF activation. This response then overwhelms and overrides the material's impact on the contact pathway activation, making it play a minor role in the extent of observed thrombus.
- D. **Time/incubation period:** The main measured response i.e., the extent of surface-associated thrombus, tends to be intense within the first 0.5–2 hrs. Sometime after this period the variable host thrombolytic (fibrinolytic) system can initiate and begin removing some of the associated thrombus.

- E. **The explant technique:** Depending on the make-up and the extent of the surface-associated thrombus on the sample, the thrombus material being measured/scored can be fragile. As such it can be dislodged during the device retrieval/sample exposure period. Moreover, without special precautions this material, often referred to as a 'sleeve thrombus', can be 'squeezed off' if the sample is retracted from or disturbed in its implant site.
- F. **The material/material surface:** This model has been used to assess the thrombogenic potential of new materials, new process modifications on existing approved materials, and to qualify new vendors. Often a predicate FDA-approved device or material is used as a control. It is not uncommon that a predicate and/or FDA-approved material/device fails the test.
- G. **Non-thromboadherent materials get labeled non-thrombogenic:** Extensive work by experts in the field (Ratner 2000, Hoffman *et al.*, 1982, and Llanos and Sefton, 1993) has demonstrated that hydrophilic surfaces can be extremely thrombogenic (and thromboembolic) yet not be thromboadherent. This test will score devices/materials that are thrombogenic yet non-thromboadherent as passing.
- H. **Recipient/subject thrombotic potential can vary remarkably:** There have been numerous papers published in this area, for example, see Kaplan *et al.*, 1986, and Brummel-Ziedens *et al.*, 2005 and 2009. Scientific evidence supports that individual test subjects can have different 'thrombotic potentials' i.e., differing capacity to form thrombus upon exposure to stimuli. Thus, test scores will be highly dependent on the test subject's thrombotic potential.
- I. **Statistical power:** Animal use requirements outlined in ISO 10993 and response variation make obtaining a statistically-meaningful conclusion from NAVI testing difficult or unethical.
- J. **Evaluator expertise:** The training and skill of the evaluator assigning thrombus scores is extremely important, as some may have difficulty differentiating between true *in vivo* thrombus and post/ante-mortem (agonal) clot formations.

Despite its controversies, the NAVI model has shown some utility. It has been useful in helping to characterize the non-thrombogenic/non-thromboadherent properties of heparin coatings on catheter-type devices. It has also proven to be a reliable method to purposely grow acute thrombus to study thrombus properties, and to examine thrombus effects on specialized devices such as sensors intended for acute or chronic blood placement (see [Plate VII](#) in color section between pages 246 and 247).

8.7 Conclusion and future trends

Almost two decades since his original editorial 'The blood compatibility catastrophe' (Ratner, 1993) the well-respected scientist Buddy Ratner has followed up with a second editorial (Ratner, 2007) speculating on why progress has been so slow in evolving a clear understanding of blood compatibility. This chapter appears to address one of his hypotheses for the slow progress: that we understand some of the biology behind blood compatibility but we do not understand how to evaluate or test for it. Looking back, indeed we cannot congratulate ourselves for making tremendous progress in this area. Advances in cell and molecular biological have put a host of tools at our fingertips to assess and measure important reactions of medical devices and materials upon contact with blood. And, advances in the fields of biochemistry, chemistry and polymer science have given us ideas and means to alter actual blood-contacting surfaces to improve responses without affecting favorable bulk material properties. To be sure, progress has been slow. Yet not to be too harsh, we should remember the famous quote by Oliver Wendell Holmes: 'The great thing in the world is not so much where we stand, as in what direction we are moving.' We are moving in some positive directions.

Through the helpful participation of numerous subject matter experts (SME) from industry, academia, and governmental groups, the guiding standard *ISO 10993-4 Biological evaluation of medical devices: Selection of tests for interactions with blood* is presently undergoing extensive revision. While these efforts generally focus at making the standard easier to understand and apply, they aptly include specific efforts to help bring refined methods, new methods, and resolution to certain controversies. In the area of standard hemolysis testing, a round robin re-validation study comparing ASTM, NIH, and MHLW (Ministry of Health, Labour and Welfare) (Japan) methods, conducted on a small subset of known materials, is being considered. The goal is to show that each method, regardless of blood source (animal vs. human) gives consistent results across low-, medium-, and high-hemolytic materials. Consistent results will support the transposable use of these methods (and test data) and help avoid excessive, wasteful, and redundant testing. In the area of complement testing on medical devices and materials, SMEs are being consulted and literature searches performed in an effort to shed some light on the 'why/what/how/how much' to test for regarding complement. Emphasis will be on how to bring about a thorough and meaningful evaluation of safe device/device material responses in this area. Factors such as which complement fragments to test for, optimal test conditions (e.g., ideal anticoagulant(s), blood preparation method(s)), and the types and sizes of devices that should require such testing are being re-addressed.

The area of testing devices and device materials for thrombogenicity is also receiving significant attention. Up front is an appreciation that the

current subdivisions of test categories into thrombosis, coagulation, platelets, hematology and complement activation is not without limitations, as each has some overlap in evaluation for thrombogenicity. Moreover, as a prominent test requirement, hemolysis testing fails to get its own category in the current ISO 10993-4, and it carries a low level of confidence as an indicator of overall hemocompatibility. In an effort to describe ‘test categories for consideration’ in a more sensible order, starting with *in vitro* testing followed by *in vivo* tests, new arrangements such as that described in Fig. 8.12 are being discussed.

The lack of one or more standard and reliable *in vitro* models to assess device material thrombogenicity is also driving dialogue on improvements. Discussion here is focused on assessing the interest in, and a proper study design for, a round robin study on this topic. Suggestions have been made for comparing several simple models, along with anticoagulation level, well-known materials, donor, and study center as variables in a full-factorial study design. Modern molecular tools would be used to gauge responses, for example, thrombin-antithrombin ELISA assay and fibrinopeptide A ELISA to estimate coagulation activity reflective of underlying thrombosis. These (and other) indicators of thrombosis, tested using standardized conditions may shed some light on the reproducibility and predictivity of *in vitro* tests to reflect *in vivo* phenomena.

Finally, it could be mentioned that ISO 10993 standard (ISO 10994-1, 2009) states that validated and reliable *in vitro* test methods ‘shall be considered for use in preference to *in vivo* tests’ and that ‘whenever possible, *in vitro* screening shall be carried out before *in vivo* tests are commenced.’ This policy is clearly intended to minimize the use of *in vivo* testing. An *in vitro* test that would replace or minimize use of the NAVI model would be consistent with this policy and clearly welcomed in the field. The efforts in ISO 10993-4 described here are consistent with efforts in other parts of ISO 10993 that are focused on upgrading and enhancing biological evaluation of medical devices with methods that are robust, at the scientific forefront, and predictive of human safety.

8.8 Sources of further information and advice

Many of the references cited are excellent sources of further reading in the various topics. An excellent source for further information will be the soon-to-be-published *Biomaterials Science: An Introduction to Materials in Medicine*, 3rd Ed.; Ratner, B. D., Hoffman, A. S., Schoen, F. J., and Lemons, J. E., Eds.; Elsevier Academic Press, San Diego, California. This is expected to be released in early 2013. This text book will have updated chapters by SME in blood coagulation and blood–material interactions, complement biology, *in vitro* and *in vivo* assessment of tissue compatibility, and

| Device examples | Test category | | | | | | | | | | |
|--------------------------------|------------------|--------------------|-------------|-----------|------------|------------|--------------------|-------------|-----------|------------|------------|
| | Hemolysis | In vitro studies** | | | | | In vivo studies*** | | | | |
| | ASTM, NIH, Japan | Thrombosis* | Coagulation | Platelets | Hematology | Complement | Thrombosis | Coagulation | Platelets | Hematology | Complement |
| External communicating devices | | | | | | | | | | | |
| Device A | x | tbd* | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd |
| Device B | x | tbd* | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd |
| Device C | x | tbd* | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd |
| Implant devices | | | | | | | | | | | |
| Device X | x | tbd* | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd |
| Device Y | x | tbd* | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd |
| Device Z | x | tbd* | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd | tbd |

tbd = to be determined.

*Thrombosis is classically defined as an *in vivo* phenomenon. However, appropriately designed mock-physiological flow *in vitro* models may produce acute thrombus-like material consistent with that seen *in vivo*.

** Validated models must be thoroughly described; protocols must provide details on which/how test categories will be assessed; discussion on validation studies and model limitations must be provided.

*** *In vivo* study protocols must provide details on which/how test categories will be assessed.

8.12 One example of the proposed new layout for Tables 1 and 2 in the standard ISO 10993-4 Biological evaluation of medical devices: Selection of tests for interactions with blood.

evaluation of blood–material interactions, among other related important topics. Also, as hinted at in this chapter, the ISO 10993-4 working group participants are actively discussing the concepts of multicenter round robin studies in the areas of hemolysis testing and simple *in vitro* testing for thrombogenicity. Moving forward, the goal is to generate written finalized protocols by 2012 and to complete all testing by mid-2013. An important goal of this work is its publication for use and review by interested and practicing scientists in these areas. These publications would be expected to occur in late 2012–2013. The following resources may be of further value:

8.8.1 Alternative testing

- European Centre for the Validation of Alternative Methods
<http://ecvam.jrc.ec.europa.eu/>

8.8.2 Standards

- American National Standards Institute
<http://www.ansi.org/>
- ASTM International
<http://www.astm.org/>
- British Standards Institution
<http://www.bsigroup.com/>
- FDA Consensus Standards, General Program Memorandum # G95-1
<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfstandards/search.cfm/>
- ISO Technical Committee 194
<http://isotc.iso.org/livelink/livelink/open/tc194/>

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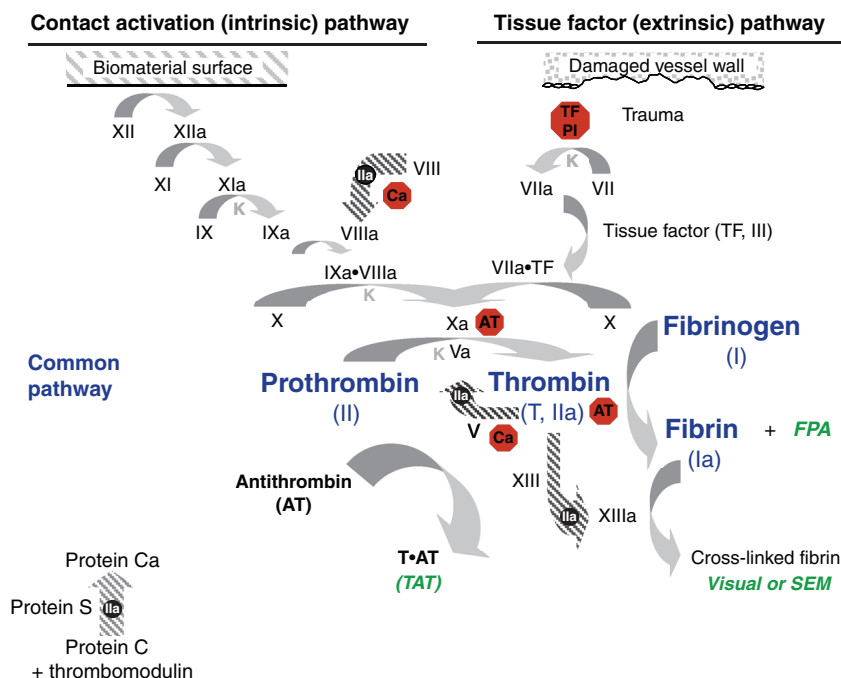


Plate I (Chapter 8) The coagulation cascade and main constituent proteins in the contact activation and tissue factor pathways. To monitor the extent of coagulation cascade activation, clinically available assays such as ELISA kits for FPA, TAT, and prothrombin fragment F1.2 (not shown) may be used. Fibrin, which can be estimated visually when laced with red blood cells, is readily seen by scanning electron microscopy. Proteins highlighted by red hexagons have a blocking or deactivation role.

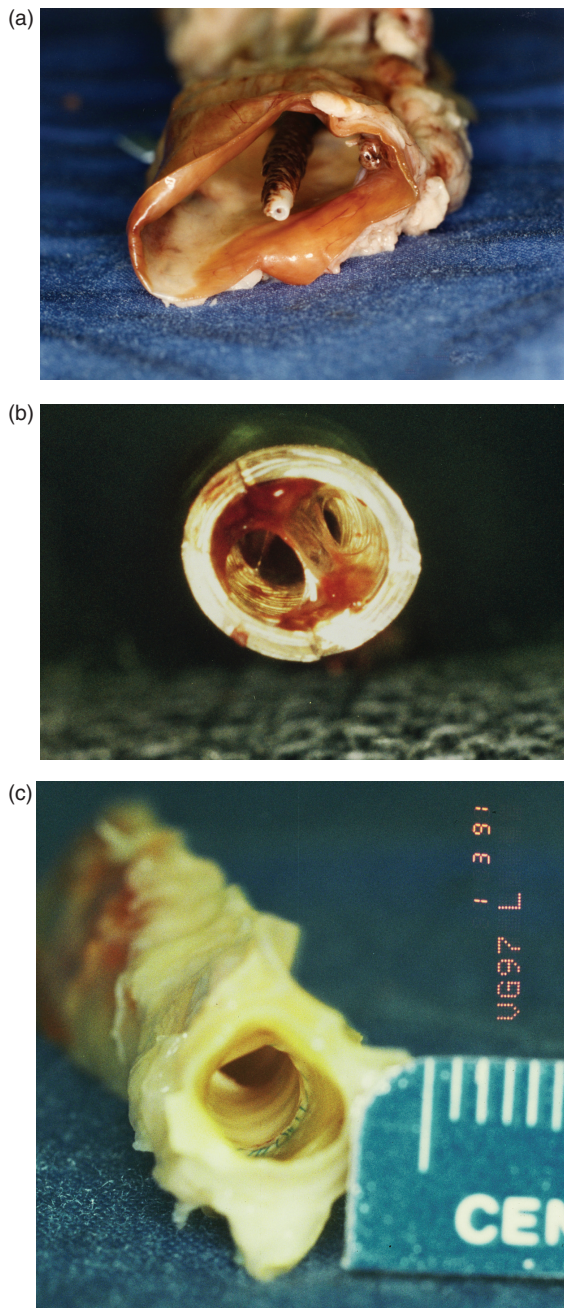


Plate IV (Chapter 8) (a) Proximal aspect looking down a vein at a polyurethane central venous catheter showing naturally occurring acute sleeve thrombus buildup. (b) Similar to panel (a) but the device is a venous cannula with a semi-occlusive web of acute thrombus. (c) View down a segment of artery containing the proximal anastomosis of a patent small diameter vascular graft.

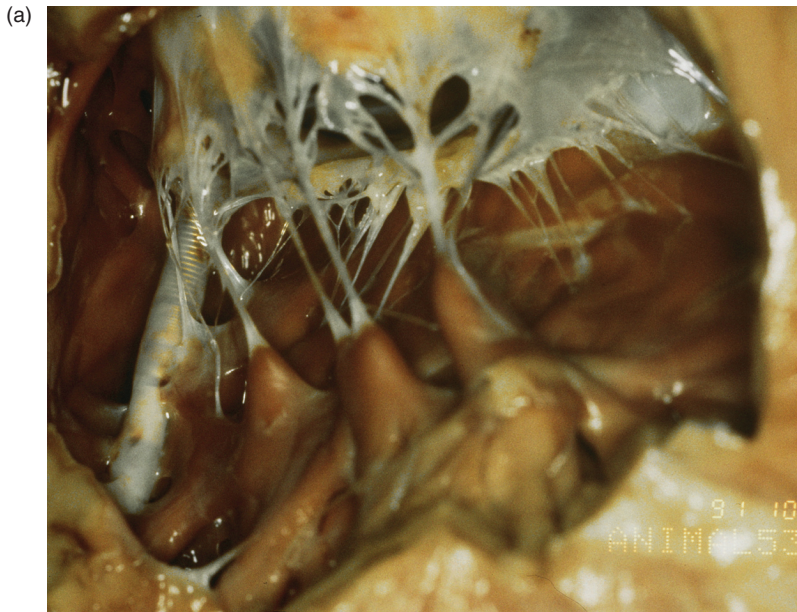


Plate V (Chapter 8) (a) Encapsulating tissue (opaque white material) on a pacing lead passing through the tricuspid valve into the right ventricle (canine model). Similar material is observed on pacing leads in the often larger and more trabeculated human hearts. (b) Same as panel (a) showing variable degrees of encapsulation tissue on two prototype intra-cardiac sensors placed in the same ventricle.

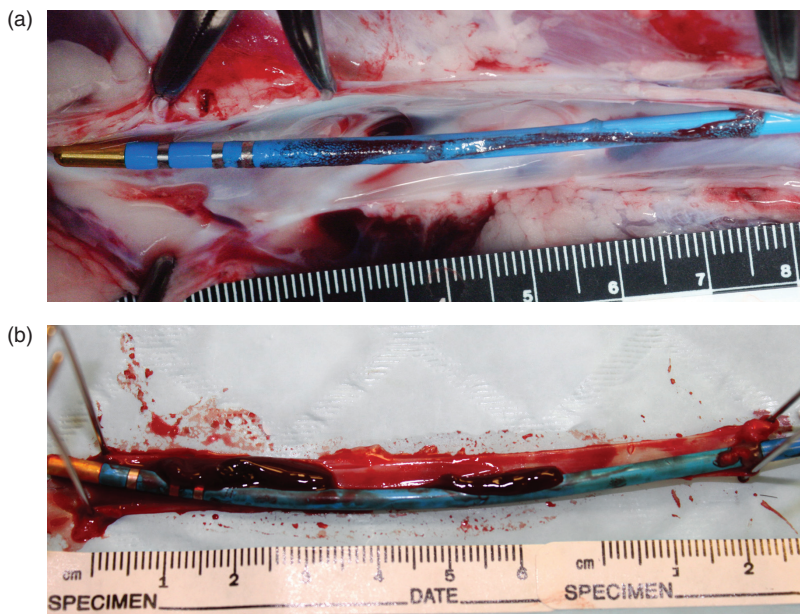


Plate VI (Chapter 8) Example results obtained using the NAVI model. (a) Results on a specialized catheter composed of multiple materials. Scoring on such a response can range from passing to failing, depending on the reviewer. (b) Results wherein a grossly apparent agonal clot was misread as *in vivo* thrombus and the device was viewed as failing the test.

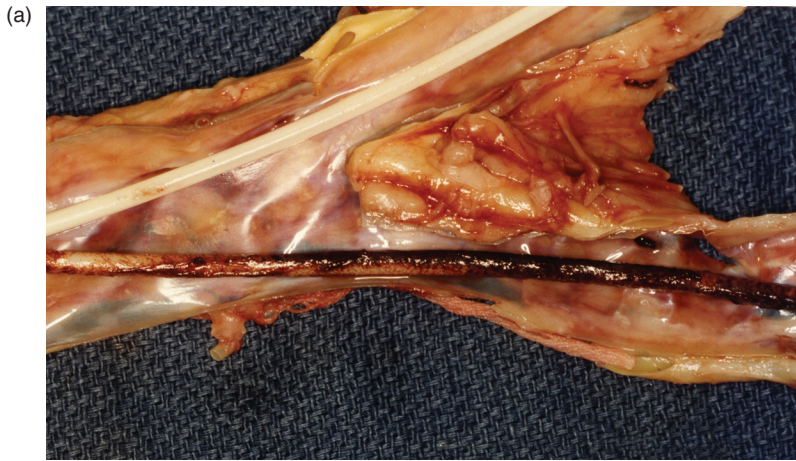


Plate VII (Chapter 8) (a) NAVI results on a heparin-coated central venous catheter. The upper device is heparin coated; the lower device is the uncoated FDA-approved control device. (b) A prototype intra-cardiac pressure sensing device purposely used in the NAVI model to study the impact of acute thrombus on sensor performance. Note that in both cases the devices presenting thrombus will receive a failing score in the NAVI test, despite their use of commonly approved materials, and highly beneficial and low adverse event clinical performance.

Medical device biocompatibility evaluation: an industry perspective

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Abstract: Determining what biological testing is required for the development and approval of a new medical device can be complex. Biological evaluation of medical devices is governed by standards such as ISO 10993, FDA Blue Book Memorandum G95-1, and Japanese Ministry of Health, Labor and Welfare Notifications and Ordinances. This chapter highlights the processes and steps involved in the evaluation of new medical devices and materials. It discusses how chemical characterization and toxicology data are used to evaluate risk and establish biocompatibility. A detailed overview of the development of biological evaluation plans and reports is described. Biological evaluations of new therapeutic products that combine medical devices with drugs or biologics are also described. Additionally, a list of sources of scientific and regulatory information is provided.

Key words: ISO 10993, biological evaluation, medical devices, chemical characterization, risk assessment.

9.1 Introduction

Evaluating the biocompatibility of medical devices and biomaterials is a complex process. This complexity arises from the fact that devices are made of a diverse range of materials for a wide variety of intended uses with body contact ranging from transient skin and blood interaction to permanent implantation. The material(s) of manufacture, the final product, and possible leachable chemicals or degradation products are all considered for their relevance in the overall toxicological evaluation of the device. Consensus standards have facilitated the biocompatibility evaluation of medical devices. In the early 1990s the International Organization for Standardization (ISO) developed a set of such standards for medical devices (ISO 10993), which provide a unified basis for medical device biocompatibility evaluation.¹ Since then these guidelines have been regularly reviewed and updated. The current controlling standard, ISO 10993-1:2009, describes the biological evaluation of any material or medical device intended for use in humans as part of a structured biological evaluation program within a risk management

process.² Subsequently, specific guidance on the conduct and interpretation of individual tests has been provided in ISO 10993 subparts 2–20. In 1995, the United States Food and Drug Administration (FDA) issued its Blue Book Memorandum G95-1, outlining modifications to the ISO 10993 test matrix that suggest additional tests for some device categories based on their potential risk.³ More recently, the FDA recognized the ISO 10993-1:2009 standard with a detailed list of exceptions.⁴ The Japanese Ministry of Health, Labor and Welfare has also developed its own guidelines for biological evaluation of medical devices which incorporate some ISO 10993 recommendations.⁵

The role of ISO 10993-1 is to serve as a framework for biological evaluation planning within a risk assessment process. Key aspects of that process are chemical characterization, toxicological, risk assessment, and biological safety testing. Chemical characterization is done to identify the constituents of the material prior to risk assessment and biological testing. Chemical constituent identification is strongly emphasized in ISO 10993-1:2009 and ISO 10993-18:2005.⁶ Toxicological risk assessment is also a central tenet of the bioevaluation process. Risk assessment results may identify the need for additional chemical analysis or lead to the conclusion that biological safety tests are unnecessary. The final step in the process is biological safety testing. It involves both *in vitro* and *in vivo* methods. Because it may help minimize the numbers of test animals, *in vitro* testing is preferred when it yields information equally relevant to that obtained by *in vivo* methods.

It should be noted that biological evaluations also include a review of manufacturing processes and relevant clinical data. Such an evaluation could result in the conclusion that it may not be necessary to conduct all of the ISO 10993 recommended biological tests if the device is made of chemically well-characterized materials that have a long history of safe use in a specified role and physical form that is equivalent to that of the device under design. If a manufacturer chooses not to conduct some of the proposed tests, however, it should document in its regulatory submissions the use of similarly processed equivalent materials in legally marketed devices with comparable patient exposures.

This chapter presents an industry perspective on biomaterials evaluation, discusses future trends, and provides information sources that will help medical device developers meet regulatory requirements, increase safety and effectiveness, and facilitate materials selection decisions. These issues are addressed in the following sections.

9.2 Developing a biological evaluation plan

For medical devices to be clinically acceptable they must be biologically safe. Biological safety is critical to the patient, the manufacturer, and regulators.

For manufacturers, biological safety evaluation is a complex process because it takes into account medical device design, use, application, materials selection, and production processes. It is a procedure that concerns biological and chemical interaction with patients, not mechanical malfunction.

A biological safety evaluation is a structured approach for documenting the biocompatibility of a medical device or material that is intended to directly or indirectly contact a patient. This process, shown in Plate VIII (see colour section between pages 246 and 247), is guided by ISO 10993-1:2009.⁷

The bioevaluation process steps depicted in [Plate VIII](#) are detailed in the following sections.

9.2.1 Initiation

The biological evaluation process is typically initiated by requests from product development, manufacturing, or regulatory affairs. Those requests are often triggered by one of the following events:

1. new device,
2. new material,
3. design change,
4. process change,
5. supplier change.

An initial meeting is frequently held with the requestor and biomaterials specialists to discuss the project. Depending upon the manufacturer's size, the staff responsible for biomaterials evaluation may reside in a business or in a centralized corporate staff group. Meeting attendees include design engineers, materials scientists, and manufacturing engineers, regulatory affairs specialists, toxicologists and biomaterials study directors. At this meeting the requestor may provide product samples, instructions for use, processing details, materials characterization data, Material Safety Data Sheets (MSDS), technical data sheets, analytical testing results, etc., and history of clinical use information.

9.2.2 Plan development

A biomaterials study director creates a Biological Evaluation Plan using information provided by the requestor to determine if biological safety testing is required. The Biological Evaluation Plan addresses the applicable sections of ISO 10993-1:2009 that pertain to biological effects and any agency-specific requirements such as those presented in FDA's G95-1 Blue Book Memorandum.⁸

9.3 Implementing a biological evaluation plan

After a biological evaluation plan has been prepared, it needs to be implemented in a systematic fashion. Steps involved in this process include: (1) materials characterization, (2) historical data collection, (3) history of safe use evaluation, (4) analytical chemistry testing, (5) toxicological risk assessment and (6) biological safety testing. Guidance covering these steps is provided by ISO consensus standards. In the following subsections steps 1 through 5 are reviewed along with a summary of key ISO standards. Biological safety testing is discussed later in Section 9.4.

9.3.1 Materials characterization

A key first step in the biological evaluation process is materials characterization (see Chapter 5). ISO 10993-1:2009 requires that chemical constituents of biomaterials or devices be addressed. Constituent information may come from a variety of sources including the supplier/vendor/manufacturer, internal or external analytical labs, device/material master files, published literature, product specification files, etc. If chemical constituent data is not available, then new analytical testing may be required.

Collectively, knowledge of the material's composition including additives and processing aids, prior use of the material in a predicate device or similar device, and biological safety data should provide predictive evidence of any potential hazard to patients.

In recent years many manufacturers have switched from paper files to electronic databases for archiving chemical and biological testing reports. Such databases have helped simplify and streamline the materials characterization procedure.

9.3.2 Vendor and historical data

Historical testing information may come from internal laboratories, external contract research organizations (CROs), or from vendors. When historical testing is cited, a comparison of past and present processing should be completed. This comparison may be included in the Biological Evaluation Plan (preferred for single material evaluations) or separate Processing Comparison memos may be written as part of the biological evaluation process (preferred for multiple material evaluations). These documents are referenced in the Biological Evaluation Plan/Report. They document whether differences exist between the processing or supplier of the material/component in the historical testing and the material/component being evaluated along with the potential impact of these differences on biological safety.

9.3.3 History of safe use

Biological safety testing may not be necessary for certain devices made from well-characterized materials with long histories of safe use. This is more often true for metals and ceramics than for polymers. Files containing history of safe use information are typically kept by regulatory affairs and/or quality assurance departments. These files contain reports from hospitals, doctors, patients, and others that document safety issues in the field. When combined with sales data they provide a qualitative and quantitative safety summary for the device.

Prior to finalization, the study director may circulate the plan for review and/or hold a meeting with subject matter experts (e.g., analytical chemists, toxicologists, materials scientists, regulatory specialists, veterinary surgeons, or pathologists).

The Biological Evaluation Plan may state that no additional testing, rationale, or review is required. Alternatively it may recommend that one or more of the following be performed:

- analytical chemistry testing;
- toxicological risk assessment;
- biological safety testing.

9.3.4 Analytical chemistry testing

If a material's composition is not verifiable, if processing or vendors have changed, or if historical test data is inadequate, then additional analytical testing may be necessary. The goal of such testing would be to detect and quantify unknown constituents, processing residues, additives, leachable substances, and degradation products (see Chapter 5). A wide variety of analytical techniques are available to accomplish this goal.

ISO 10993-18:2006. 'Biological Evaluation of medical devices – Part 18: Chemical characterization of materials' provides guidance on performing chemical characterization tests on the material, component or device in question. Chemical characterization testing helps in identifying and evaluating a material's chemical constituents. These constituents may include metals, monomers, antioxidants, additives, colorants, process aids, cleaning solvents, and mold release agents.⁹

ISO 10993-18:2006 provides a comprehensive list of tests for analysis of polymers, metals, alloys, ceramics and natural macromolecules.¹⁰

Polymer tests include gas and liquid chromatography, mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction, non-volatile residue, inductively coupled plasma (ICP) and X-ray photoemission spectroscopy (XPS).

Tests for metals and alloys include X-ray fluorescence, combustion analysis, gas fusion, atomic absorption, ICP, titrimetric, gravimetric, electrolytic, colorimetric, X-ray diffraction, electron dispersive spectroscopy (EDS or EDX), XPS and metallography.

Tests for ceramics include X-ray fluorescence, ICP, ion chromatography, colorimetric, X-ray diffraction, and microscopy.

Tests for natural macromolecules include colorimetric, 2D PAGE, molecular weight analysis, NMR, FTIR, amino acid analysis and sequencing, titration, thermal analyses, gas and liquid chromatography, and dialysis.

9.3.5 Toxicological risk assessment

Risk assessment is a systemic scientific evaluation of potential adverse health effects resulting from human exposures to hazardous agents or situations.¹¹ Risk is defined as the probability of an adverse outcome based upon the exposure and potency of the hazardous agents. Risk assessment requires an integration of both qualitative and quantitative scientific information.¹² The four components of a risk assessment are:

1. *Hazard identification*: An evaluation of the basic health risk presented by an agent of interest. Examples might include the capacity of a chemical to cause lung cancer or liver damage.¹³
2. *Dose-response assessment*: A quantitative determination of the amount of agent needed to cause a toxic reaction along with an estimation of the dose that is likely to produce little or no adverse effect.¹⁴
3. *Exposure assessment*: An appraisal of the source, type, magnitude, and duration of contact with the agent. A key step is determining which exposure pathways are the most relevant.¹⁵
4. *Risk characterization*: An analysis and integration of pertinent information from the hazard, dose-response, and exposure assessments that describe the assumptions and uncertainties involved and justify confidence in the conclusions.¹⁶

Information on previous uses of each intended additive or leachable constituent and any adverse reactions encountered should be reviewed. Account should be taken of the intended use, the concentration of constituents, and toxicity data from animals and/or humans. Much of this data is available from Internet-based toxicology databases and government reports (see section 9.7 Sources of further information and advice).

A risk assessment may conclude that exposure to the material(s) in question does not pose a health risk to the patient. If so, then the bioevaluation is complete. If not, then biological safety testing may be indicated.

9.3.6 Regulatory guidance

The following four ISO consensus standards provide guidance and information for conducting toxicological risk assessments:

ISO 14971:2007. 'Medical devices – Application of risk management to medical devices' provides guidance with respect to evaluation of biological hazards.¹⁷ Annex I, 'Guidance on Risk Analysis Procedure for Biological Hazards' offers direction regarding hazards due to chemical constituents with the potential for causing biological harm. According to this consensus standard, in order to estimate risk, three major factors must be taken into account:

1. chemistry of the hazardous material;
2. prior use of this material;
3. biological safety data.

ISO 10993-1:2009. 'Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process' provides guidance on conducting toxicological risk assessments. It states that risk assessments shall consider the specific nature and duration of exposure: chronic toxicity, carcinogenicity, biodegradation, toxicokinetics, immunotoxicity, reproductive and developmental toxicity, and other organ-specific toxicities.¹⁸

ISO 10993-7: 2008. 'Biological evaluation of medical devices – Part 7: Ethylene oxide sterilization residuals' provides examples on calculating allowable exposure limits.¹⁹

ISO 10993-17:2002. 'Biological evaluation of medical devices – Part 17: Establishment of allowable limits for leachable substances' provides guidance on calculating tolerable intake (TI) values for non-cancer and cancer endpoints, tolerable exposure, and tolerable contact levels.²⁰ For prolonged exposures (>24 h–30 days) this ISO standard indicates that subchronic toxicity values such as No Observed Adverse Effect Level (NOAEL) or No Observed Effect Level (NOEL) are preferable for calculating TI values. However, for permanent exposures (> 30 days), chronic toxicity data (NOAEL or NOEL) are preferred. If available, human data is preferred for establishing a margin-of-safety and identifying potential toxicological risks.

9.4 Biological safety testing

Depending upon the results of the toxicological risk assessment, biological safety testing may be required. ISO 10993-1:2009, FDA Blue Book Memorandum G95-1, and ASTM F748-06 provide general principals and advice on test selection.^{21,22} The types of testing are determined by the nature

and duration of tissue contact (see Chapter 12). Commonly conducted tests include:²³

- cytotoxicity,
- irritation,
- sensitization,
- acute systemic toxicity,
- subacute and subchronic systemic toxicity,
- implantation,
- hemocompatibility,
- genotoxicity.

Rarely conducted tests include:

- chronic toxicity,
- carcinogenicity,
- reproductive and developmental toxicity,
- biodegradation,
- neurotoxicity,
- immunotoxicity,
- toxicokinetics.

9.4.1 Contract research organizations

Instead of maintaining internal biological safety laboratories, many medical device firms have outsourced this testing to CROs. In the US, Europe, and Asia there are a number of CROs that specialize in biocompatibility testing (see Information Sources). Most provide biological safety testing, analytical analysis, and consulting services. These laboratories are well equipped and staffed by professionals knowledgeable about international standards and country-specific regulations.

Working with such outside labs is a straightforward process. Manufacturers either determine the tests they wish to conduct or seek advice from the CRO about appropriate testing. Once the test plan has been finalized the manufacturer will prepare samples and ship them to the CRO by overnight delivery. In turn the CRO will conduct the tests under Good Laboratory Practice (GLP) conditions and prepare a signed report, which is delivered to the manufacturer electronically.²⁴

9.4.2 Testing and sample preparation

Factors that need to be considered when biological safety testing is planned include the type and size of samples to be tested along with extraction procedure parameters. These issues are discussed in the following sections.

Materials versus device testing

ISO 10993-1:2009, and some global regulatory agencies, recommend that biological safety testing be performed on the assembled, processed, and sterilized final product. This is to ensure that all raw materials, additives, and manufacturing processes are evaluated.

The standard, however, also states that testing may be performed on representative samples of the final product or on materials processed in the same manner as in the final product. Therefore, another way that biological evaluations may be documented is to provide biological safety data for the separate tissue-contacting materials and/or components, which comprise the device. Under this approach, the materials should be processed as closely as possible to the final product, or documentation should include comparison of the processing of the test article to the processing of the final product, identification of any processing differences, and justification that any processing differences are not expected to affect the biological safety of the final device.

The final device testing approach has the advantage of being more straightforward and can aid in streamlining the regulatory submission process. However, testing performed on a composite device usually cannot be used in support of the biological safety of any of the individual constituent materials or components in a subsequent device. The ‘separate materials’ approach may draw additional regulatory scrutiny, but it does afford some benefits. Testing on a separate material or component may potentially be applied (with proper processing evaluation) to the use of that material or component in subsequent devices.

Extracts and sample size

ISO 10993-12:2007. ‘Biological evaluation of medical devices – Part 12: Sample preparation and reference materials’ offers guidance on sample size and extraction conditions appropriate for biological safety testing.²⁵ These conditions are exaggerated in time and temperature to provide a margin-of-safety over normal physiological conditions. Solvent recommendations are also provided. For most devices and in most geographies, only a polar (e.g., saline) and non-polar (e.g., vegetable oil) solvent are required to simulate hydrophilic and hydrophobic physiological fluids.

9.4.3 Biological evaluation report

After executing the Biological Evaluation Plan the study director creates a biological evaluation report that summarizes findings from the chemical characterization, toxicological risk assessment, and biological testing phases of the evaluation. The report may incorporate documents such as history of use files,

analytical results, and the risk assessment. Once completed, the report is circulated to stakeholders for review, then updated, signed, and archived.

9.5 Creating a biological evaluation report

At the end of the biological safety assessment process, the available test data and rationales, toxicological risk assessment, and other related documents on biological safety of the materials, components and devices need to be assembled into a biological evaluation report. Such a report can be submitted to worldwide regulatory agencies to support product registration. Thus it is important for it to include sufficient evidence to demonstrate full compliance with ISO 10993-1:2009.

9.5.1 Elements of a biological evaluation report

The format of the biological evaluation report varies. It may contain the following information as applicable:

1. *Descriptions of the device and components:* Including product information, intended use, and type and duration of patient contact of the material and device. Drawings of the device and component that help illustrate the patient contact may also be included.
2. *Chemical characterization of the device materials:* The material composition or formulation information can sometimes be obtained from material suppliers. However, such information is often proprietary. So it is not uncommon for the device manufacturer to sponsor or perform chemical analytical studies to identify and characterize the materials and leachables in the devices.
3. *Manufacturing and processing information:* Examples include molding, curing, and processing additives, along with cleaning, sterilization, and primary packaging chemicals or reagents.
4. *Biological safety information needs:* This is based on the recommended evaluation tests for consideration by the applicable international or national standards and guidelines such as ISO-10993-1:2009 and FDA G95-1. Each test requirement should be addressed by relevant test data or rationales.
5. *Biological safety test data sources:* Device manufacturers or materials suppliers may sponsor biological safety studies. GLP guidelines are applicable for biological safety studies that are used to support product registration. Safety study test results and procedures should be briefly summarized in the biological evaluation report. Full safety study reports should be included with the biological evaluation report. Sometimes materials or components may have been tested before, individually or

as part of a device. If so, the existing data may be used to address test requirements of a new device. An experienced person with appropriate training and experience should review all test data to determine their applicability.

6. *Rationale for selecting specific tests:* This includes sample size, extraction conditions, sterilization methods, chemical analysis methods, etc. These are especially critical for deviations from the normal conditions.
7. *Additional relevant data:* There may be published studies or reports on the biocompatibility of materials with long histories of safe use. The biological safety of these materials can also be shown by their history of use in existing devices with comparable patient contact. Information from literature searches and clinical experience may add to the available biological safety testing data.
8. *Toxicological risk assessment:* Certain tests may not be necessary if existing data or published literature adequately show the specific health risk to be low. The risk assessment process has been discussed in detail in other sections of this book.
9. *Conclusion:* A discussion of any deviations from the biocompatibility assessment protocol, and rationale of whether such deviation is acceptable or not. A summary statement that confirms the biological safety of the device for its intended use.
10. *Review and approval signatures:* This may include, but is not limited to, the biocompatibility assessor, R&D, quality assurance, regulatory affairs, project management, and production departments.
11. *Change history of the materials and processes:* As discussed in detail in Change Management below.

An example of a bioevaluation report is provided in the information sources section at the end of this chapter.

9.5.2 Change management

During the device's life cycle, the design, materials, and manufacturing processes frequently change. If any patient contact material is affected by such changes then the impact on biocompatibility of the device must be considered. According to ISO 10993-1:2009, the materials or final product shall be re-evaluated if any of the following occurs: (a) any change in the source or in the specification of the materials used in the manufacture of the product; (b) any change in the formulation, processing, primary packaging, or sterilization of the product; (c) any change in the manufacturer's instructions or expectations concerning storage, for example, changes in shelf life and/or transport; (d) any change in the intended use of the

product; or (e) any evidence that the product may produce adverse effects when used in humans.⁷

If the sources, manufacture specifications, or process specifications of the patient contact materials and components of the devices have been altered, then it is important for the biomaterials study director to determine the effect of such change on the materials. Many manufacturing steps such as thermal, mechanical, and chemical processes may have significant impact on the device and its materials. For example, heat or cleaning solvents may destabilize polymers and adhesives, or increase leachables from the devices; processing reagents such as lubricants, abrasives and releasing agents also leave residues or significantly affect the chemical and physical properties of the material. The primary packaging materials may produce leachables during sterilization processes that affect biocompatibility of the product.

When material or process changes are brought to the biomaterials study director's attention, the first step is to determine if the chemical properties of the patient contact materials are affected. One should obtain as much detail as possible about the difference between the new and previous formulations or processes. Chemical or analytical studies can be used to compare the new and previous materials. A toxicological risk assessment can be performed based on known chemical information of the material. Based on the analysis, it may not be necessary to repeat the whole battery of biocompatibility tests if the change is minor and does not cause additional health risk to the patient. However, a simple screening test such as a cytotoxicity study may be beneficial in providing baseline data on the changed material.

It is important to document the changes to materials and processes in the biological assessment. All rationales and additional test data need to be retained with the assessment. Meanwhile, when using existing data on a particular material to meet the biological evaluation requirements for a new device or new intended use, change history and supplier information needs to be reviewed to determine the applicability of such data.

9.6 Conclusion and future trends

During the coming decade the \$300 billion global medical device industry is expected to continue experiencing steady growth. Aging populations, health insurance expansion, developing markets, innovative new products, and global harmonization of standards and regulatory requirements will all help to drive this growth. The US, Europe, Japan and Canada are large mature medical device markets with relatively low (3–5%) annual growth rates. Therefore, to accelerate their sales, medical device makers are increasing their focus on developing countries such as China and India.²⁶

In order to stay ahead in a highly competitive marketplace, medical device firms are exploring a wide variety of options for developing new

therapies, increasing innovation, improving products, and reducing regulatory approval times.²⁷ Several of these options are discussed below.

9.6.1 Combination products

Using medical devices to deliver drugs or biologics represents a relatively new therapeutic approach. Such combination products are often more effective than traditional treatments because they allow for targeted drug delivery that frequently uses less drug and produces fewer side effects. Drug-eluting stents, inhalation devices and drug-delivery pumps are examples of combination products that deliver targeted treatments locally instead of systemically. These products offer therapeutic and financial advantages by providing patients with more effective and efficient medical treatments.²⁸

Development of combination products requires close collaboration between pharmaceutical and medical device companies. Joint development is necessary because of the complexity of the products. Besides the technical challenges, other obstacles impact the development of combination products, including fundamental cultural differences between the two industries, and, mostly, the ambiguous requirements of regulatory agencies.²⁹ To facilitate timely approvals, product developers need to communicate with regulatory bodies very early in the development stage regarding biological safety evaluation, otherwise delays are inevitable due to uncertainty surrounding drug versus device regulatory control.

9.6.2 New materials

Materials science is a dynamic field the products of which are critical to innovation in the medical device industry. Therefore it is crucial for materials engineers to stay informed about the most recent advances in materials technology.

New materials need to meet challenging performance, size, biocompatibility, and biostability requirements. For example, in recent years there has been a marked increase in interest in nanoparticles and biodegradable polymers for use in medical devices.

Nanoparticles are attractive due to their unique properties of optical transparency, controllable porosity, chemical inertness and biocompatibility. Developing nanoparticles for targeted drug delivery is an area of intense research activity. One important application is to deliver poorly soluble drugs across the blood–brain barrier.³⁰

Engineered biodegradable polymer materials are attracting attention because of their capacity to hold and slowly deliver drugs in a controlled release fashion as they degrade *in situ*.^{31,32}

9.6.3 Alternative testing

Toxicology testing is nearing a scientific pivot point. Advances in biology and biotechnology may transform traditional animal-based toxicology testing to a discipline founded primarily on *in vitro* methods that use human cells and computer modeling.³³ This new paradigm would make toxicity testing quicker, less expensive, and more relevant to human exposures.^{34,35}

The concept of the 3Rs – Reduce the use of animals, Refine experiments to minimize distress and pain, and Replace animals with alternative techniques – is often cited as the origin of the move towards animal-free toxicity testing. The principles of the 3Rs were developed by Universities Federation for Animal Welfare (UFAW) scholars, Professor William MS Russell and Rex Burch at the direction of Major Charles Hume, founder of the UFAW. Major Hume proposed that the UFAW undertake a scientific study on humane treatment of laboratory animals. This study led to publication in 1959 of the classic book *The Principles of Humane Experimental Technique*.³⁶ Since then the 3Rs have been increasingly adopted worldwide and have resulted in a significant reduction in the use of certain *in vivo* methods.³⁷

In recent years two European regulations have been key drivers pushing toxicology towards this pivot point. These are the Cosmetics Directive and the Registration, Evaluation, Authorization and restriction of Chemicals (REACH) law. On March 11, 2009 a Cosmetics Directive amendment outlawed the use of animals in seven mandatory toxicity tests. These were tests for skin irritancy, sensitivity to light, corrosivity, skin absorption, genetic toxicity, eye irritancy and acute toxicity. The amendment also banned the import of cosmetics containing ingredients that have been animal tested in this way after the deadline.³⁸ REACH went into effect on December 18, 2006. Amendment 7 of REACH encourages the reduction or elimination of *in vivo* testing, and promotes the use of other *in vitro* (e.g., High Throughput Screening (HTS)) and *in silico* modeling (e.g., quantitative structure–activity relationship (QSAR)) techniques.^{39,40} In response, start-up companies, cosmetics makers, biotech firms, and others have been working hard to develop alternative test methods.⁴¹

Public interest in alternative testing is growing worldwide and governments are taking notice. The European Centre for the Validation of Alternative Methods (ECVAM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the USA are both involved in validating and promoting the use of novel test methods.^{42,43}

The medical device industry already uses some *in vitro* methods for biocompatibility testing (e.g., cytotoxicity, genotoxicity, and hemocompatibility). Other current animal tests such as irritation, sensitization, thrombogenicity, and pyrogenicity may also be amenable to *in vitro* methods. If pilot projects currently underway are successful, then ECVAM and/or ICCVAM will most probably be recruited to validate their accuracy.

In the years ahead the device industry will undoubtedly strive to reduce the number of animals used in toxicity testing. Public opinion and international standards will both help drive this move. As the ISO 10993-1:2009 standard states: '*In vitro* test methods, which are appropriately validated, reasonably and practically available, reliable and reproducible *shall* be considered for use in preference to *in vivo* tests.'⁴⁴

9.6.4 Safety evaluation

ISO 10993-17:2002. 'Biological evaluation of medical devices – Part 17: Establishment of allowable limits for leachable substances' states that: 'the determination of the suitability of a medical device for a particular use involves balancing any identified risks with the clinical benefit to the patient associated with its use. Among the risks to be considered are those arising from exposure to leachable substances arising from medical devices.'⁴⁵ It also provides a method to calculate the allowable limits to evaluate the risks associated with exposure to hazardous leachable substances.

For extractable chemicals where the chemical structure and/or quantity of the compound is known, but toxicological information is limited or impractical to obtain, other approaches such as the threshold of toxicological concern (TTC) are being considered. AAMI/ISO Working Group 11, which is responsible for ISO 10993-17, is developing a proposal regarding the use of TTC for assessing biocompatibility of extractable substances from medical devices.⁴⁶

In addition to ISO, other standards-developing organizations are also developing recommendations for evaluating leachable substance risks, optimizing processes, and choosing materials that protect patient health. For example, the Product Quality Research Institute (PQRI) has issued a recommendation entitled 'Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products' for pharmaceuticals and their various container, closure, and delivery systems.⁴⁷ The PQRI has also evaluated thresholds and best practice concepts for characterizing container closure systems with respect to leachable substances, and their associated impact on the safety of parenteral and ophthalmic drug products.⁴⁸

9.7 Sources of further information and advice

This section contains sources of information to help guide the biological safety evaluation of materials and medical devices.

9.7.1 Alternative testing

- AltTox
<http://www.alttox.org/>

- AltWeb
<http://altweb.jhsph.edu/>
- Center for Alternatives to Animal Testing (CAAT)
<http://caat.jhsph.edu/>
- European Centre for the Validation of Alternative Methods (ECVAM)
<http://ecvam.jrc.ec.europa.eu/>
- Institute for *In Vitro* Sciences (IIVS)
<http://www.iivs.org/>
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
<http://iccvam.niehs.nih.gov/>
- Japanese Center for the Validation of Alternative Methods (JaCVAM)
<http://jacvam.jp/en/>

9.7.2 Books

- Black, J. *Biological Performance of Materials: Fundamentals of Biocompatibility*, 4th ed. Boca Raton, Florida: CRC Press, 2005; 520 pp.
- Ratner, B. D., Hoffman, A. S., Schoen, F. J. and Lemons, J. E., eds., *Biomaterials Science: An Introduction to Materials in Medicine*, 2nd Ed. San Diego, California: Elsevier Academic Press, 2004; 864 pp.
- Klaassen, C. D., ed., *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 7th ed. New York: McGraw-Hill, 2008; 1280 pp.
- Gad, S. C. and McCord, M. G., *Safety Evaluation in the Development of Medical Devices and Combination Products*, 3rd ed. New York: Informa Healthcare, 2008; 300 pp.

9.7.3 Contract laboratories

- American Preclinical Systems
<http://www.americanpreclinical.com/>
- CeeTox, Inc.
<http://www.ceetox.com/>
- Nelson Laboratories, Inc.
<http://www.nelsonlabs.com/>
- North American Science Associates, Inc.
<http://www.namsa.com/>
- Toxikon, Inc.
<http://www.toxikon.com/>
- WuXi AppTec, Inc.
<http://www.wuxiapptec.com/>

9.7.4 Industry publications

- *European Medical Device Technology*
<http://www.emdt.co.uk/>
- *Massachusetts Medical Devices Journal*
<http://www.massdevice.com/>
- *Medical Device & Diagnostic Industry Magazine*
<http://www.mddionline.com/>

9.7.5 Journals

- *ALTA—Alternatives to Laboratory Animals Biomaterials*
- *Critical Reviews in Toxicology*
- *International Journal of Biomaterials*
- *International Journal of Toxicology*
- *Journal of Applied Biomaterials*
- *Journal of Biomaterials Science, Polymer Edition*
- *Journal of Biomedical Materials Research*
- *Journal of Neurotoxicology*
- *Journal of Toxicology and Environmental Health*
- *Neurotoxicology, Regulatory Toxicology, and Pharmacology*
- *Regulatory Toxicology and Pharmacology*
- *Toxicological Sciences*
- *Toxicology and Applied Pharmacology*
- *Toxicology In Vitro*
- *Toxicology Letters*
- *Toxicology Reviews*

9.7.6 International regulatory and government agencies

- Australian Therapeutic Goods Administration
<http://www.tga.gov.au/>
- European Commission – Medical Devices
http://ec.europa.eu/consumers/sectors/medical-devices/index_en.htm/
- European Medicines Agency
<http://www.ema.europa.eu/>
- Global Harmonization Task Force
<http://www.ghtf.org/>
- Health Canada
<http://www.hc-sc.gc.ca/>
- Japanese National Institute of Health Sciences
<http://www.nihs.go.jp/english/index.html>

- Japanese Ministry of Health, Labor, and Welfare
<http://www.mhlw.go.jp/english/>
- Korea Food and Drug Administration (KFDA)
<http://eng.kfda.go.kr/index.php>
- State Food and Drug Administration, P.R. China (SFDA)
<http://eng.sfda.gov.cn/WS03/CL0755/>
- United Kingdom Medicines and Health Products Regulatory Agency
<http://www.mhra.gov.uk/>

9.7.7 US regulatory and government agencies

- Food and Drug Administration (FDA)
<http://www.fda.gov/>
- FDA Center for Biologics Evaluation and Research (CBER)
<http://www.fda.gov/BiologicsBloodVaccines/default.htm>
- FDA Center for Devices and Radiological Health (CDRH)
<http://www.fda.gov/cdrh/>
- FDA Center for Drug Evaluation and Research (CDER)
<http://www.fda.gov/cder/>
- FDA Office of Combination Products (OCP)
<http://www.fda.gov/CombinationProducts/default.htm>

9.7.8 Free online databases

There are a number of excellent sources of chemistry and toxicology data available on the Internet. Free access is provided by government agencies. Key databases include:

- Agency for Toxic Substances and Disease Registry (ATSDR)
<http://www.atsdr.cdc.gov/toxpro2.html>
- ChemSpider
<http://www.chemspider.com/>
- European Chemical Substances Information System
<http://esis.jrc.ec.europa.eu/>
- OECD Existing Chemicals Database
<http://webnet.oecd.org/hpv/ui/Search.aspx>
- International Agency for Research on Cancer (IARC) Monographs
<http://monographs.iarc.fr/ENG/Classification/index.php>
- International Chemical Safety Cards (ICSCs)
<http://www.cdc.gov/niosh/ipcs/icstart.html>
- International Programme on Chemical Safety (IPCS)
<http://www.inchem.org/pages/search.html>
- US Environmental Protection Agency's Integrated Risk Information System (IRIS)

- http://www.epa.gov/ncea/iris/search_keyword.htm
- US National Library of Medicine's PubChem
<http://pubchem.ncbi.nlm.nih.gov>
- US National Library of Medicine's PubMed
<http://www.ncbi.nlm.nih.gov/pubmed>
- US National Library of Medicine's TOXLINE
<http://www.nlm.nih.gov/pubs/factsheets/toxlinfs.html>
- US National Library of Medicine's TOXNET
<http://toxnet.nlm.nih.gov/index.html>

9.7.9 Fee-based online databases

There are a wide variety of proprietary databases available that contain toxicology research reports, health & safety data, and MSDSs. They are accessible over the Internet from vendors such as Dialog and STN. Leading sources include:

- BIOSIS® Toxicology
<http://library.dialog.com/bluesheets/html/bl0157.html>
- Canadian Centre for Occupational Health & Safety (CCOHS)
<http://ccinfoweb.ccohs.ca/>
- Dictionary of Substances and Their Effects (DOSE)
http://www.knovel.com/web/portal/browse/display?_EXT_KNOVEL_DISPLAY_bookid=527
- Embase
<http://www.embase.com/info/what-is-embase/coverage>
- International Pharmaceutical Abstracts (IPA) Toxicology
<http://library.dialog.com/bluesheets/html/bl0153.html>
- MSDS-OHS
<http://stneasy.cas.org/dbss/help.MSDS-OHS.html>
- Patty's Toxicology Online
<http://onlinelibrary.wiley.com/book/10.1002/0471125474>
- Registry of Toxic Effects of Chemical Substances (RTECS)
<http://www.cdc.gov/niosh/rtecs/RTECSfeatures.html>
- SciSearch
<http://library.dialog.com/bluesheets/html/bl0034.html>

Note: Due to their complexity and cost it is advisable that professional help be sought for searching proprietary databases.

9.7.10 Scientific associations

- American College of Toxicology
<http://www.actox.org/>

- Australian Society for Biomaterials and Tissue Engineering
<http://www.biomaterials.org.au/>
- Canadian Biomaterials Society
<http://www.biomaterials.ca/>
- European Biomaterials Society
<http://www.esbiomaterials.eu/>
- EUROTOX
<http://www.eurotox.com/>
- Japanese Society of Biomaterials
<http://wwwsoc.nii.ac.jp/jsbm/>
- Korean Society of Biomaterials
<http://www.ksbm.or.kr/>
- Society of Biomaterials
<http://www.biomaterials.org/>
- Society of Toxicology
<http://www.toxicology.org/>

9.7.11 Standards

- American National Standards Institute
<http://www.ansi.org/>
- ASTM International
<http://www.astm.org/>
- British Standards Institution (BSI)
<http://www.bsigroup.com/>
- FDA Consensus Standards
<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfstandards/search.cfm/>
- International Organization for Standardization (ISO)
<http://www.iso.org/>
- ISO Technical Committee 194
<http://isotc.iso.org/livelink/livelink/open/tc194/>
- United States Pharmacopeia (USP)
<http://www.usp.org/>

9.7.12 Trade associations

- Association for the Advancement of Medical Instrumentation
<http://www.aami.org/>
- AdvaMed
<http://www.advamed.org/>
- EucoMed
<http://www.eucomed.be/>

- LifeScience Alley
<https://www.lifesciencealley.org/default.aspx/>

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9.9 Appendix: example of a material component biological evaluation report template

Material/Medical Device Name
Biological Evaluation Report

Document Number XXXX
Version 1.0

1.0 Introduction

The introduction contains descriptions of the material or medical device including categorization according to ISO 10993-1 and other applicable standards for type and duration of contact. The name and model number of the material or device should be included along with an explanation of its use. Drawings or pictures of the device may also appear here.

2.0 Chemical characterization

This section covers chemical characterization information on the materials from which the device is comprised. These include CASRN, physicochemical properties, vendor data, and analytical testing results.

3.0 Manufacturing processing

This part provides a description of the relevant manufacturing process steps conducted by the supplier or manufacturer, including cleaning, sterilization, and packaging. A list of all processing aids, additives, and reagents should be included.

4.0 Tissue contact

Details concerning the nature and duration of a material or device's contact with the patient are presented here.

| Material/ part number | Device/ part number | Contact category | Tissue contacted | Contact duration |
|--------------------------|------------------------|--|---|---|
| Polymer A No. XXXX | Catheter B No. XXXX | Surface, externally communicating, or implant device | Mucosal membrane, breached or compromised surface, tissue, bone, blood, etc. | Limited, prolonged, or permanent |

5.0 Evaluation of ISO 10993-1 compliance

This section discusses how biological safety testing results and other pertinent information on the material or device complies with the ISO 10993-1 standard and its subparts. The following table provides an example of how such information might be summarized.

| Biological effect | Test methods | Reference | Results |
|---|--|---|-------------------------------|
| Cytotoxicity | Cytotoxicity test (MEM elution) | ISO 10993-5: 2009, Tests for cytotoxicity | Pass/fail |
| Sensitization | Maximization sensitization test (guinea pig) | ISO 10993-10: 2010, Tests for irritation and sensitization | Sensitizer/non-sensitizer |
| Irritation or intracutaneous reactivity | Intracutaneous/intradermal test (rabbit) | ISO 10993-10: 2010, Tests for irritation and sensitization | Irritant/non-irritant |
| Systemic toxicity (acute) | Systemic toxicity (mice) | ISO 10993-11: 2006, Tests for systemic toxicity | Pass/fail |
| | Material mediated pyrogenicity (rabbit) | ISO 10993-11: 2006, Tests for systemic toxicity) | Pass/fail |
| Subacute and subchronic toxicity | Subchronic toxicity test; 14-day repeat dose (rats) | ISO 10993-11: 2006, Tests for systemic toxicity) | Pass/fail |
| Chronic toxicity | See ISO 10993-1:2009, Section 6.2.2.10 | ISO 10993-11: 2006, Tests for systemic toxicity) | Pass/fail |
| Genotoxicity | Reverse mutation test (bacterial cells) | ISO 10993-3: 2003, Tests for genotoxicity, carcinogenicity, and reproductive toxicity | Genotoxic/non-genotoxic |
| | <i>In vitro</i> mammalian chromosome aberration test (Chinese hamster ovary cells) | ISO 10993-3: 2003, Tests for genotoxicity, carcinogenicity, and reproductive toxicity | Genotoxic/non-genotoxic |
| | Micronucleus assay (mice) | ISO 10993-3: 2003, Tests for genotoxicity, carcinogenicity, and reproductive toxicity | Genotoxic/non-genotoxic |
| Carcinogenicity | See ISO 10993-1:2009, Section 6.2.2.11 | ISO 10993-3: 2003, Tests for genotoxicity, carcinogenicity, and reproductive toxicity | Carcinogenic/non-carcinogenic |

(Continued)

| Biological effect | Test methods | Reference | Results |
|---|--|---|---|
| Reproductive and developmental toxicity | See ISO 10993-1:2009, Section 6.2.2.12 | ISO 10993-3: 2003, Tests for genotoxicity, carcinogenicity, and reproductive toxicity | Toxic/non-toxic |
| Implantation | Intramuscular implant in rabbits (12 weeks) | ISO 10993-6: 2007, Tests for local effects after implantation | Gross and microscopic findings |
| Hemocompatibility | Hemolysis, <i>in vivo</i> thrombogenicity, and complement activation | ISO 10993-4: 2002, Selection of tests for interaction with blood | <i>In vitro</i> and <i>in vivo</i> findings |

5.1 *Test sample size, preparation, summary of test results as appropriate*

5.2 *Toxicological risk assessment findings and references*

5.3 *Any additional relevant data*

6.0 Discussion

Discuss any deviation from the Biological Evaluation Plan and provide justifications.

7.0 Conclusions

This section provides a summary of the biological information on the material/component. It should include a statement that the findings in this report demonstrate compliance of the material or medical device with the applicable sections of ISO 10993-1 and/or other country-specific standards.

8.0 References

9.0 Attachments

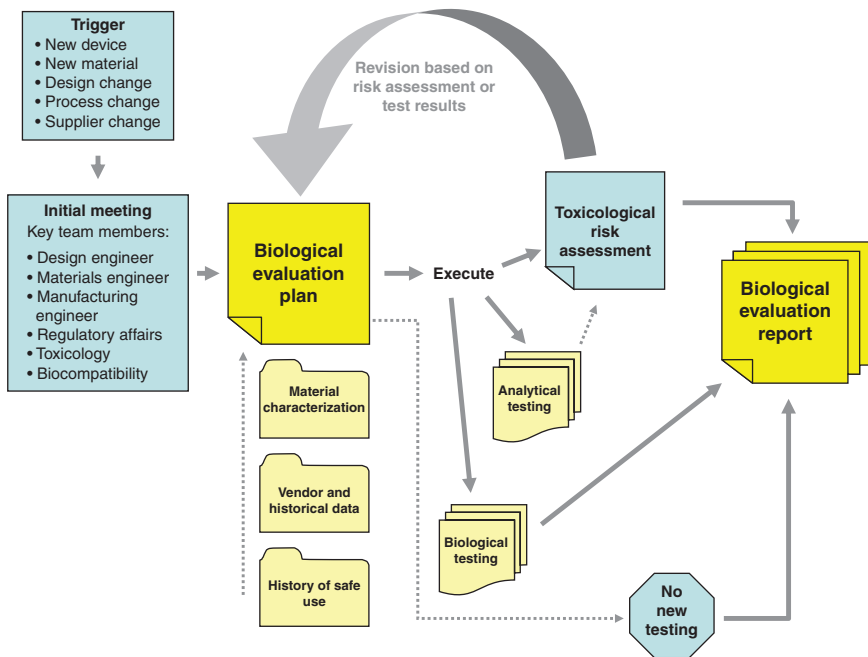


Plate VIII (Chapter 9) The bioevaluation process.

Case study: overcoming negative test results during manufacture

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Abstract: Designing the biological safety program is our opportunity to define risk control. However, if our biological safety program is solely based on testing, it is then solely reactive. I test and discover that my product is likely biocompatible or has some issues that could compromise biological safety. I have a program based on pass or fail. I have wasted time and money only to find out that I have a problem. This is not the best approach. Biological safety is a control program. Therefore it is a feedback loop mechanism. The control of adverse events is based on intended use, the materials of construction, and the manufacturing materials and processes. Biological safety must be designed into the product. Equal emphasis must be placed on the materials and the process relative to intended use.

Key words: risk, biological safety, biocompatibility, manufacturing.

10.1 Introduction

There are several steps or precautions that can be built into a manufacturer's standard operating procedures that will help minimize the potential for adverse findings in biological safety studies. The first step is driven by the mindset of the manufacturer and the culture that persists through the medical-device firm making the product. A mindset must exist that says that 'any material used by my company is not biocompatible unless I demonstrate that my material is biocompatible.' The other truism the manufacturer must adopt is that 'in the absence of data our hypothesis remains unproven.'

I often make live training presentations and one of the things that I consistently preach during these presentations is to 'Know your place in the World.' More simply put, this means that we need to understand how we are perceived relative to the world in which we work. On the most basic level, we work in the medical-device industry. Our products improve the public health. They are regulated by government agencies and other regulatory bodies such that the right of law or the privilege to access markets only belong to those who indeed know their place in the world, and understand the critical nature of manufacturing such products. We need to understand that our products are primarily opportunities to improve lives and more

broadly the public health, before they are opportunities to improve the bottom line. While the firm's existence may be based on the latter, excellence is only reached when emphasis is placed on the former.

So what does this have to do with controlling adverse findings? It is the recognition that making a product using the same materials as another product, or the same materials that have been used in devices before, does not constitute sufficient manufacturing controls to control adverse findings. It is the recognition that our products demand far more scrutiny than consumer goods. It is the recognition that deliberate planning is necessary. Planning must examine all factors that can contribute to biological hazards. This typically boils down to:

- intended use,
- materials of construction,
- production process including materials of manufacturing.

10.2 Cardio Medical: a fictitious case study

Take the case of Cardio Medical, a fictitious company that I often use in training sessions, which is launching an intravenous catheter line of 'ultra-thin' catheters that allow for maximum lumen size given the circumference or gauge of the catheter. Cardio is in the design phase. The product has been prototyped. The manufacturing process has been defined and preliminary lists of both the materials of construction and the manufacturing materials used in the process have been assembled. Table 10.1 shows us this fictitious list, containing only the major materials for this exercise.

The device is a short-term device with blood contact. Since it is not a permanent implant, it is not a high-risk device but nonetheless it maintains contact with circulating blood. Therefore, any chemicals made available by the device could enter the patient's bloodstream. The blood can then carry potential toxins, carcinogens, etc. to target organs and other areas of the body.

Table 10.1 Materials of construction for intravenous catheter

| Component | Material |
|---------------------------------|--|
| Catheter | DuPont teflon |
| Introducer | 304 stainless steel |
| Coating | Silicone medical fluid |
| Manufacturing material: ethanol | Solvent for applying the silicone medical fluid |
| Hub | Ultra-high molecular weight polyethylene (UHMWPE) |
| Hub color | Varies with size, that is, 20 g is yellow, 21 g is green, etc. |

10.3 The biological safety program

Designing the biological safety program is our opportunity to define risk control. However, if our biological safety program is solely based on testing, it is then solely reactive. I test and discover that my product is likely biocompatible or has some issues that could compromise biological safety. I have a program based on go or no-go. I have wasted time and money only to find out that I have a problem. This is not the best approach.

Biological safety is a control program. Therefore it is a feedback loop mechanism. In order to control we must first measure, we must then understand, analyze, put steps in place to maintain control and confirm by more measurement. Chemical analysis and *in vitro* biological assays are our main tools early on to make sure that our materials, processes, and design are designed with biological safety in mind. We must understand the contribution that materials, manufacturing, and intended use have on the biological safety of our product. It is not sufficient to simply test for biological safety – we must build safety into the product.

Returning to our catheter, it is important to determine what chemical extractables are available from the product. This is also somewhat controversial as in medical-device extraction scenarios described in ISO 10993-18, polar and non-polar solvents, including solvents of intermediate polarity such as ethanol, are used to conduct chemical analysis. For extractables testing, we repeat extractions so that we can exhaustively determine any chemical species that can potentially be removed from the product. In the drug industry, extractables represent anything that can be removed (typically from a drug package) under abusive conditions. That being the case, solvents can be modified so that they have an abusive effect on the polymer thus causing it to swell and perhaps deteriorate.

At this stage it is important to gain information about our product so that we can manage the risk as we design production and continue to plan for biological safety. We would want to make sure that the coating used on the introducer is safe, that there is little residual alcohol to cause an adverse effect, that the color does not bleed from the hub and what does that colorant make available to the patient. We would not expect issues with polytetrafluoroethylene (PTFE) but we want to make sure that our studies challenge the potential use of these materials.

10.4 Extractables and leachables

The information we gain from extractables testing is invaluable as it enables us to view the worst-case, though improbable, scenario for the patient. Extractables testing introduces us to risk but does not represent a practical occurrence. For that we must look at leachables, that is, a chemical

species that actually migrates during the use of the device. If, when examining extractables we can satisfactorily mitigate risk then determination of leachables may not be necessary. In fact, it is quite desirable to demonstrate that the levels of extractables are safe, so that there are no concerns about particular what-if scenarios that could invalidate the extraction technique that may have been used for leachables.

10.5 Controlling risk at the manufacturing level

Indeed these potential risks can be mitigated through further manufacturing changes. These changes could involve material substitutions but they can also involve processing changes. In our example, a dip into the coating/alcohol mixture may be modified to a spray. Or perhaps the solvent is replaced in favor of one that is more favorable to patient safety. The result of the extractable and leachable studies allows us to begin to establish a safety profile for the device and its materials. It is this safety profile that forms the basis for risk assessment and it is the risk assessment that provides us with the analysis to establish risk control.

Risk control can be applied in two different directions. We can further improve the safety profile through additional manufacturing changes or we can reduce the risk by conducting tried and true biological test methods that allow us to feel more certain about the safety of the device. Either way we bridge the gap required to reach the level of risk necessary to assume biocompatibility. Or do we? We only can use testing to aid this situation if we have sufficient manufacturing controls in place to demonstrate that the test results are indeed valid.

Another example of a manufacturing process that can influence the biological safety of a device is the cleaning process. By this we mean that the use of a cleaner is necessary to remove debris, lubricants, oils, and other processing aids from a device or a component as it is being manufactured. This process introduces some complexity into the manufacturing process as we must demonstrate that the cleaner is effective in removing whatever it is we have used to manufacture the device, and we must provide assurance that cleaning residues are reduced to levels that will then be safe to the patient.

Let us say we are a dental manufacturer selling dental burrs. These are those lovely devices used in our mouth that the majority of us despise when we are having a filling placed in a cavity or perhaps preparing what is left of a tooth for a crown. The primary manufacturing process used for dental burrs is machining and frankly there is little difference in machining a dental burr from a mechanical burr, despite the small size of the dental burr and the quality of material used. A machining process leaves us with debris (particles), oils, and perhaps other lubricants. These contaminants then have to be removed by another potential contaminant, the cleaner. Cleaners tend to

be more toxic than other materials or processing aids used in manufacturing so while we must subject our dental burrs to the cleaning process, we must then take steps to remove the cleaner to safe levels. A rinsing process needs to be developed and validated to minimize the levels of residual cleaner remaining, or to reduce the amount of cleaner to levels that are safe.

ISO 10993-17 was developed for this purpose. This ISO document is used to measure the allowable limits of leachable substances. The document was modeled on the 1995 work done on ethylene oxide (EO) residuals. This work gave rise to ISO 10993-7. This document replaced the FDA draft 21CFR821 section on concentration limits for EO residuals. The draft CFR has since been removed and the latest revision of ISO 10993-7 stands at 2008.

While 10993-17 was developed as a guide to calculate these limits, it reads very much like ISO 14971, annex I. The latter document uses the calculations as a part of risk assessment. ISO 10993-17 tries to avoid the risk assessment label, often unsuccessfully. In the case of our example cleaner it tends to do both. It can be used to set the limit for the cleaner, but the limit is based on risk as expressed by the term margin of safety or margin of exposure. These terms refer to the ratio of the amount of cleaner (in this case) available for patient exposure to the allowable limit for the cleaning agent.

$$\text{Margin of safety} = \frac{\text{Exposure (amount available)}}{\text{Allowable limit (per ISO 100993-17)}}$$

The document can then be used to institute manufacturing controls that will keep the residual cleaner within safe limits when used.

10.6 Sterilization residuals

Sterilization is a common process for many medical devices that can cause potential biological safety issues. The most obvious are the acceptance criteria laid out in ISO 10993-7. By controlling levels of EO residuals, we can then control the risk of adverse biological effects arising from this process. While we are concerned about the biocompatibility of biomaterials, the use of EO is probably the single most toxic event that we can implement in the manufacture of a device. It is proof that a manufacturing process can have a significant effect on the biological safety of a medical device.

Manufacturing controls can be put into place to help control sterilization residuals such as EO. The cycle itself can be optimized so that we are using the minimum amount of gas necessary to achieve the desired sterility assurance level (SAL). Chambers can be designed with dynamic air washes that assist in removing the gas very efficiently. The aeration phase of the process can be modified to allow for greater aeration efficiency after the product is removed from the sterilizing chamber and placed into aeration.

Sometimes design controls are equally important to manufacturing controls. If the product can be designed so that it is easier to sterilize, then a combination of less gas or less exposure can be used in order to minimize the resulting residuals.

EO and its companion, ethylene chlorohydrin (ECH), are just two of the residuals that are of concern. TC 194 WG11 is working on a Technical Report for dealing with DEHP, a common plasticizer that has fallen out of favor due to its biological and environmental hazards. Other sterilization residuals, such as formaldehyde and gluteraldehyde, may follow in the future. These all stress the need for appropriate material selection and manufacturing process controls.

10.7 Conclusion

The control of adverse events is based on intended use, the materials of construction, and the manufacturing materials and processes. All three make an equal contribution to the safety of the product. Biological safety must be designed into the product and must be evaluated in terms of risk. The emphasis most firms place is on the materials used to manufacture the product. Often the manufacturing process is forgotten. Adverse events can be controlled by understanding the nature of risk that each of the three have to product safety. Risk control is our key tool. Just remember to examine the role of manufacturing when deciding how risk should be dealt with.

Methods for the characterisation and evaluation of drug–device combination products

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Abstract: This chapter describes methods that can be used in the characterisation and evaluation of drug–device combination products. A brief definition for combination products and an outline of how these are regulated are provided. Demonstration of safety and efficacy is discussed, together with description of a range of pre-clinical testing that should be considered for such products. Some considerations for manufacturing aspects and clinical trial design are also presented, together with perspectives on the future outlook for combination products and their evaluation.

Key words: combination product, pre-clinical testing, *in vitro* bench testing, biocompatibility and toxicity testing, safety and efficacy studies.

11.1 Introduction to combination products

In order to significantly advance patient care, innovative technologies are required that will bring about a step-change in the performance of the medical products of the future. Combination products offer one approach to convey such a promise. They comprise two or more regulated components (e.g. drug–device, biologic–device or drug–biologic) either physically, chemically or otherwise united in one product, in an effort to provide synergistic benefits from their combined properties. These products come in a wide variety of different formats, for instance combined in the packaging as pre-filled syringes containing drug or biologic, metered dose inhalers, transdermal patches, drug-laden wound dressings, or implantable combinations such as surgical sealants or drug-eluting stents (DES). In some cases (as in the US) they may still be classed as combination products even if the two components are co-packaged, such as for needleless injectors or delivery pumps plus a drug–biologic sold in the same pack or separately packaged and cross-labelled.

Combination products are not new and have in fact been around since the Medical Devices Amendments of 1976; but it is only since the turn of the millennium that some regulatory bodies have provided guidance to industry on the development of these products. This has been driven primarily

from the increased activities of both the medical-device and pharmaceutical industries in creating products primarily for implantation that are capable of drug delivery for the local treatment of specific biological sequelae or disease states. The therapeutic agent may, for instance, ameliorate an adverse biological response that ensues as a consequence of the implantation or presence of the device; for example, an anti-inflammatory agent delivered from endocardial pacemaker electrodes is added to reduce fibrous connective tissue formation, resulting in the need for lower voltage stimulation thresholds and also aiding in its eventual removal from the body.¹ This approach therefore overcomes deficiencies in the device performance related to biocompatibility or the body's reaction to the implant, which cannot be overcome by changes in the engineering design alone. Similarly, the active agent may be selected to guard against any subsequent infection following placement of the device (as for antibiotic-containing orthopaedic cements²). Alternatively, the active agent can be present to address a pre-existing disease, as is the case for embolic drug-eluting beads (DEBs); arteries feeding a tumour are primarily physically occluded by intra-arterial administration of the device, which can then subsequently deliver a sustained and local dose of chemotherapy to the diseased and dying tissue.³ Thus, combination products can offer significant clinical benefits in terms of enhanced device performance, with improvements in drug efficacy and safety by virtue of high local concentrations and lower systemic exposure.

This chapter will discuss some of the considerations that should be made when planning the pre-clinical testing of combination products, with a specific focus on drug-device combinations.

11.2 Combination product regulation

Clearly there is an abundance of new considerations for the combination product for which the application of conventional product safety and efficacy testing, as outlined throughout this book, may be insufficient. The specific requirements may depend upon the office to which the combination product is assigned for review; each component will have a different formal regulatory pathway in itself, which will impact greatly on all aspects of the product development, approval and commercialisation. There will of course be some common ground, and typically information on laboratory testing, animal studies, stability testing, clinical study design, long-term follow-up and post-approval data may need to be provided. The perspective of the different agencies reviewing the data, however, may be quite different and hence care must be taken in consultation with all involved, on what constitutes an acceptable approach to satisfy all of the requirements. It is ultimately the developer's obligation to perform a risk assessment in order to guide the decisions as to the appropriate testing. A detailed account of the

respective regulatory requirements for combination products is therefore outside the scope of this chapter; the reader is referred to Chapters 18 (S. Leppard) and 19 (J. Greenbaum) of the book: *Drug-device Combination Products: Delivery Technologies and Applications*, which review European and US regulatory aspects in great detail.⁴

11.3 Demonstrating safety and efficacy of combination products

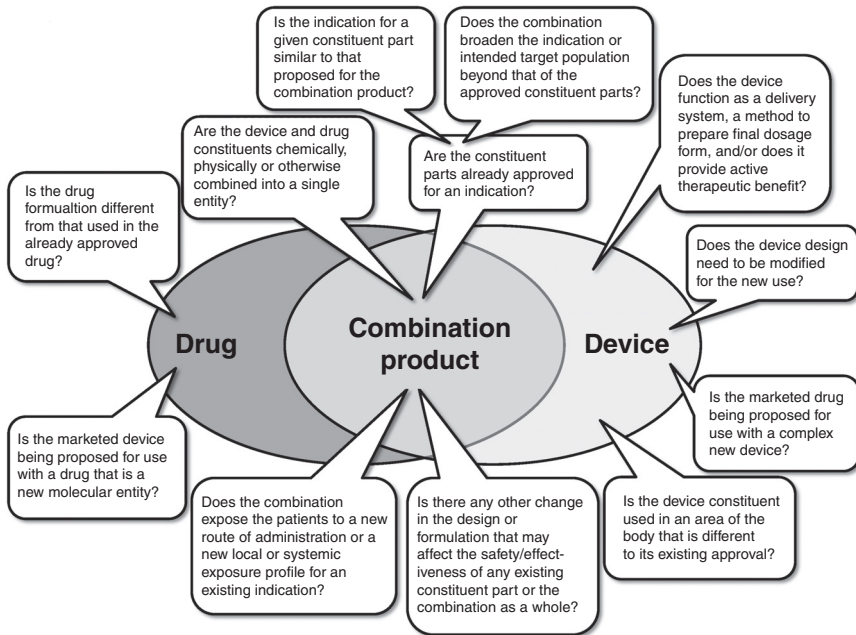
There are numerous guidance documents available to help determine the requirements for demonstrating safety and efficacy of combination products,⁵⁻⁹ but depending upon the degree of innovation of any new technology, these principles may need to be significantly adapted. Hence, the developers of such products are encouraged to consider not only the component parts but particularly the issues raised by placing them in combination. This should ensure the generation of a comprehensive and inclusive approach without the need to perform studies that become superfluous by virtue of the combination.

11.3.1 Combinations composed of one or more previously approved/cleared components

The ideal place to start with the development of a combination product is one in which one or both of the constituent parts have been previously approved and/or cleared for use in the indication for which the combination product is proposed. This avoids a great deal of testing and will streamline the development process. The key, however, is to recognise what new issues are now raised as a consequence of combining the components. Figure 11.1 illustrates some of these considerations as set out in the Guidance for Industry and FDA Staff: Early Considerations for Innovative Combination Products.⁵

Clearly, the issues may not be simply about how the components may interact when combined, but also about such matters as potential changes in the proposed use of the combination product, the route by which it is administered, changes in local or systemic exposure to drug component, or type of patient population to be treated. Indeed, a product used to treat two different indications may be physically the same in each case but classified differently depending upon its primary mode of action in each indication.

In the case where the drug component is a new molecular entity (NME), it is necessary to first execute the conventional pre-clinical evaluations required to establish its safety, followed by first-in-man studies of the NME itself, before embarking upon additional studies to assess the combination product. Careful planning is essential, as some of the tests that might be



11.1 Considerations for combination product development using one or more currently marketed components.

required, such as reproductive toxicity or carcinogenicity studies, are long-term in nature but may be undertaken in parallel with some of the early clinical investigations if the risks are appropriately mitigated.

11.3.2 Evaluation of the interactions between the combination product component parts

When combining device and drug components, there is the potential for interaction. Indeed, certain interactions may be desirable; for instance, the device may be used to control the release of an active agent in order to sustain its delivery over a prolonged period,⁴ or the combination may promote stability of the drug in its most active form.¹⁰ It is therefore important to understand the following:

- whether the drug stability is changed when combined with or delivered from the device;
- if there are physical or chemical drug–device interactions that alter the dose of the drug delivered, for example by a catalysed drug degradation or adsorption of the drug to the device;

- whether the presence of the drug interferes with the mechanical function of the device, or promotes degradation of any part of the device;
- if there are leachables or residuals from manufacturing of the device that can interact with the drug or pose a safety issue upon their release;
- whether the device has an action at the point of use that could change the characteristics of the drug (for instance, as for photodynamic therapy where this is desirable).

Hence, robust validated methods for extraction and analysis of both the drug and device components may be required in order to detect such potential changes. Chromatographic (or other separation) methods that are able to separate pure drug from impurities and degradants are traditionally used.¹¹ High pressure liquid chromatography (HPLC), for instance, is a mainstay technique that is utilised to measure both dose and purity of components with accuracy and precision. Furthermore, physical methods will be needed to measure the influence of the drug on the physicomachanical properties of the device.

11.4 Pre-clinical testing of combination products

Broadly, the pre-clinical (or sometimes referred to as non-clinical) testing of a medical device essentially evaluates safety using a number of endpoints which include the overall biocompatibility of the device (for instance, as per recognised standards such as ISO 10993), component testing (e.g., by the American Society for Testing and Materials (ASTM) methods), the appropriate selection and qualification of the raw materials used in the construction of the device and the final design validation testing prior to regulatory submission. For a drug–device combination product the pre-clinical testing package may need to be broadened in order to address new safety concerns introduced by the incorporation of the drug and the evaluation of its pharmacodynamics.

11.4.1 *In vitro* methods for bench testing

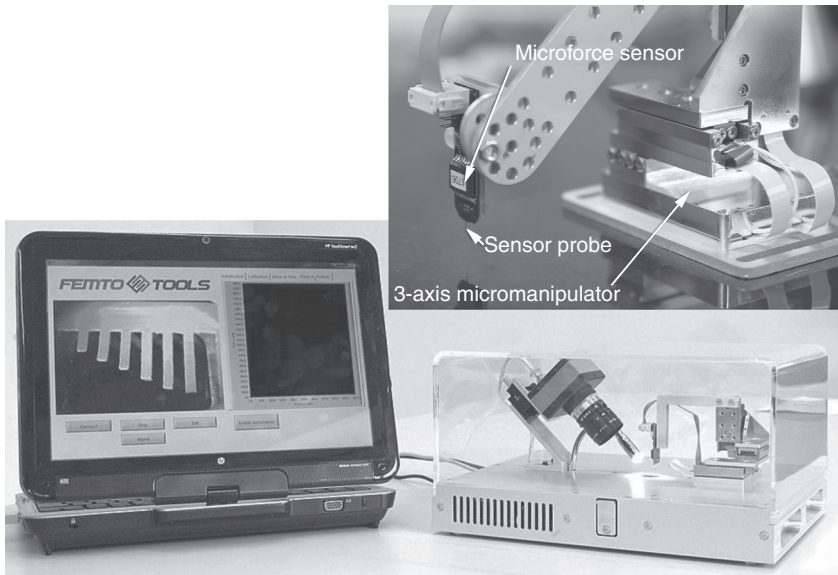
In vitro bench testing provides the first assessment of the combination product performance.^{12,13} Where the product exists in multiple configurations, for example the different lengths and diameters of DES, or the various calibrated size ranges of embolic DEBs, the amount of product testing required can quickly expand exponentially. Bracketing is a common approach to reducing the testing burden, where the extremes of a particular parameter are chosen and a justification made that everything in between is the same. This relies on the parameter either remaining constant, or varying predictably across a

range; but it may also be a risky approach if one of the extremes fails testing throwing the outcome of such a matrix in doubt. Clearly, given the myriad of sizes, shapes and uses of combination products, new bench tests may need to be developed in order to provide relevant information about the performance of the product in its proposed mode of application.

Physicomechanical testing

As drug–device combination products contain a device element that normally has a physical function, physicomechanical testing is usually required. This ensures that the device maintains its physical integrity for the duration of its expected use. In combining the drug and device elements, some adverse interaction may occur that could lead to early physical failure of the device component (as per Section 11.3.2), for instance, the inclusion of an antibiotic into an orthopaedic cement can influence how the material sets and impact on its subsequent physical ability to withstand mechanical load.^{14,15} Physicomechanical performance tests are devised to measure these effects and allow for engineering solutions to be applied to design around any issues. Conventional mechanical testing equipment, such as dynamic mechanical analysers, tensometers and indenters can be applied to measure a wide range of mechanical properties including tension, compression, flexure, fatigue, impact, torsion and hardness.

Case study: embolic DEB. The resistance to compression for embolic DEBs is an important parameter that dictates whether the product can be delivered through microcatheters, which can often possess inner lumen diameters smaller than the beads and hence require the product to deform during delivery. Moreover, once administered into an artery, the beads will travel through the vessel until they physically occlude by virtue of their size; here, if the beads deform too much they can be squeezed too distally down a vessel or potentially pass through the capillaries and into the venous circulation where they could induce non-target embolisation. Whilst the deliverability of the product is best tested using a relevant microcatheter delivery use test, physicomechanical investigations have been applied during their development using an Instron tensiometer to perform a modified test which measures the force to compress a sample of a number of beads held on a platter. This test was sensitive enough to demonstrate that drug loading significantly increased the force required to compress the beads, but was incapable of producing reproducible data for the smaller bead sizes.^{16,17} Since those early reports, new technologies have been developed, such as that offered by Femtotools GmbH that uses highly sensitive force-sensing probes capable of measuring single-bead compression across



11.2 Example of a microforce sensing apparatus for physicomaterial analysis of micro-scale devices or thin coatings. (Source: Photographs courtesy of Femtotools GmbH, Zurich, Switzerland, www.femtotoools.com.)

all sizes of product (Fig. 11.2). Moreover, testing methods have evolved to be more relevant to the product indication and measures of rate of elastic recovery are now also possible that better mimic the performance of the product *in vivo*.¹⁸

Companies such as Instron and Bose have opted to develop and commercialise a wide range of device-specific testing equipment that can be used to evaluate such properties as plunger forces for needles and syringes, fatigue and durability of DES or stent grafts, strength and stiffness of metallic wires and tubing, compression and strength of breast implants or wear and stress in orthopaedic implants. These tests are aimed at demonstrating the device component maintains its primary function, and can often be performed according to recognised ASTM guidance, some examples of which are listed in Table 11.1 for a selection of medical devices.

Evaluation of drug delivery coatings

One strategy for combining a drug with a device is by use of a polymeric coating into which the active agent can be dispersed, and from which release can be subsequently modulated. Examples of combination products that utilise this approach include coronary stents that elute anti-restenosis drugs,

Table 11.1 Selection of American Standard Test Methods for medical devices

| ASTM | Test |
|-------------------------|--|
| ASTM F382 | Flexural fatigue testing metallic bone plates and fixture devices |
| ASTM F384 | Metallic angled orthopaedic fracture fixation devices |
| ASTM F543 | Torsion testing metallic bone screws |
| ASTM F1714 | Wear of prosthetic hip designs |
| ASTM F1717 | Spinal constructs: static testing, fatigue testing |
| ASTM F2079 and F2477 | Stents, tensile strength testing |
| ASTM F2256 | Strength properties of tissue adhesives by T-peel testing |
| ASTM F2346 | Characterisation and fatigue of intervertebral disc prostheses |
| ASTM F2606 | Three-point bending balloon expandable vascular stents and stent systems |

anti-inflammatory-eluting hernia meshes or endotracheal tubes that release antimicrobial agents. Regardless of the application, the coating must be mechanically robust and durable enough to withstand cracking or delamination from the device during handling and implantation; it must also be stable, to withstand the physical rigours of the device function and to act as a reservoir for the desired period of drug release.¹² Once the drug has gone, the coating may then optionally remain as a bioinert component of the device, or biodegrade; the testing required to demonstrate safety of these two formats may therefore be quite different. The coating may consist of multiple layers that serve different purposes, such as top-coats that act as barrier layers to drug diffusion (as for the poly(butyl methacrylate) top-coat on the Cypher® DES), or as subbing layers that help to bind the coating to the substrate (for example, Parylene C is often selected for this reason). Whatever the format, the primary purpose of the coating is to act as a carrier for the active substance and release it in a controlled fashion. The evaluation of such coating systems therefore requires an understanding of each of the drug, coating and device component parts and crucially, how they interact with one-another.^{19,20}

Drug analysis

The choice of drug or drug type used in a combination product is driven by the underlying biological processes that are being addressed, in order to enhance the products performance and provide a benefit to the patient. The quantity of drug required may be a function of how much is released over time to achieve the desired pharmacological effect. As the drug is delivered locally from the device, the concentrations contained within the coating may be extremely low, particularly if the device itself is small, such as a DES. Total drug loading per device may be difficult to measure and would

normally involve extraction of the drug from the coating in a suitable solvent (which can swell the coating and dissolve the drug) followed by quantification using techniques such as HPLC or other more specific techniques.²¹ The solid-state form of the drug is an important property to consider as this may influence its overall physical properties, affecting its dispersion within the coating when applied to the device. This can have major effects on the mechanical properties of the coating, the drug-release kinetics and its long-term stability. The drug may exhibit polymorphism and the ability to control its existence in crystalline to amorphous states may offer another mechanism of controlling its release; indeed, mixing a poorly water-soluble drug with the polymer coating itself may provide for beneficial drug crystal habit modification, resulting in altered release kinetics and enhanced bioavailability. The solid-state form can be determined from films of polymer and drug that are subjected to a wide range of techniques such as differential scanning calorimetry (DSC), X-ray diffraction analysis (XRD) or polarised light microscopy. Further analysis can be conducted on the coatings themselves using methods such as atomic force microscopy (AFM), where amplitude-phase-distance (a-p-d) studies carried out in Tapping Mode™ can yield information on crystalline material embedded within the coating matrix. Moreover, a modification of AFM known as Micro Thermal Analysis is able to perform DSC with the spatial resolution of the scanning probe microscopy, allowing for rapid localised thermal analysis, such as melting point determination of an identified drug crystallite.²² Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) is a technique that has gained popularity in recent years and not only enables the identification and quantification of pharmaceutical solids in coatings²³ but also allows quantitative analysis of mixtures of drug polymorphs.²⁴ This technique platform is now available coupled with microscopy and automated sample stage handling to provide mapping capability across a large area of the study sample²⁵ (see for example the PerkinElmer® Spotlight Series FT-IR microscopes). Finally, techniques such as Dynamic Secondary Ion Mass Spectrometry (DSIMS) have been applied to the study of drug distribution within and release behaviour from polymer coatings.²⁶

Coating analysis

There are a number of key properties of drug delivery coatings that must be considered when developing a drug-device combination product:²⁷

Thickness. May determine the absolute dose of drug that can be contained within the carrier matrix and rate of drug elution; may be composed of several layers to modulate release kinetics.

Uniformity. Ensures a consistent dose of drug is delivered per unit area of the coating; surface properties may also be important for bio-

compatibility. Continuous coatings may provide protection for the underlying substrate.

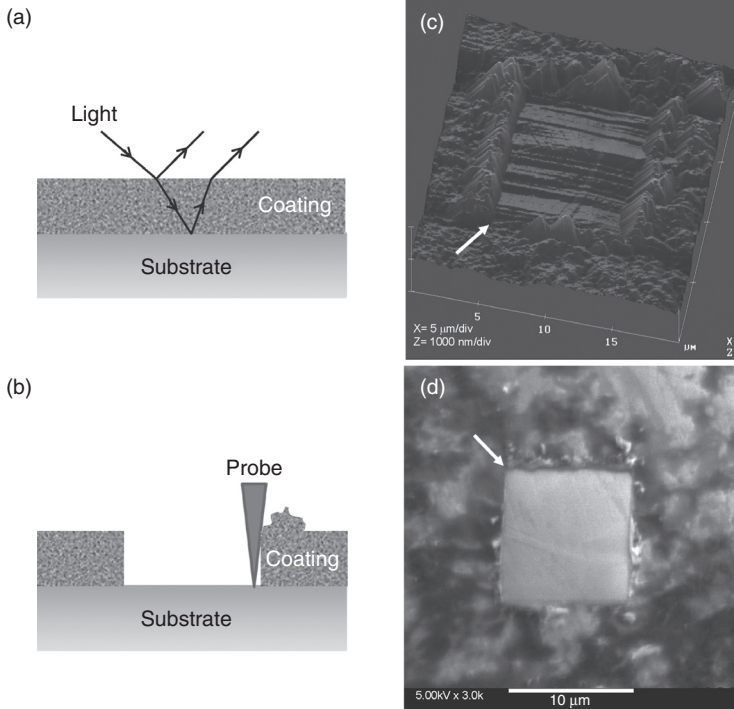
Adhesion/cohesion. Ensures the coating maintains integrity over time, is durable enough to survive device deployment and in-use stresses without flaking or delamination. Adhesion to the substrate must be firm, cohesion within the coating or between different layers is fit for purpose.

Composition. May provide mechanisms for drug interaction (charge, hydrophobic domains/phases, swelling-controlled release, biodegradation of the matrix). May determine mechanical properties (e.g. glass transition temperature (T_g) – affecting the ability of the polymer to flow and film-form, its elasticity, hardness and modulus).

There are a large number of techniques that can be used to characterise one or more of these important attributes, some of which are destructive and others which are non-destructive and potentially useful as quality control or in-line measuring tools (Fig. 11.3a and 11.3b). Of the non-destructive methods there are a number that can be usefully applied for the measurement of coating thickness, including: simple spectrophotometry or more complex spectroscopic ellipsometry, optical (white light) profilometry/interferometry, beam profile reflectometry, and confocal scanning laser or Raman microscopy. Each of these techniques has pros and cons depending upon required level of accuracy, depth resolution, multi-layer capability, translucence/refractive index properties, flat or curved geometry, amount of sample preparation, ease of use and cost considerations. Destructive techniques such as nanoindentation, focal depth indexing, stylus profilometry and some modes of AFM rely upon removal or displacement of an area of coating down to the substrate and some physical method of measuring the depth.

Case study: coronary stent coatings. AFM has been used in conjunction with scanning electron microscopy (SEM) in the analysis of explanted polymer-coated coronary stent samples to determine the presence, thickness and mechanical properties of the coating several months post-implantation^{28,29} and after release of drug into the artery²⁵ (Fig. 11.3). The one caveat to this method however, is that it requires the underlying substrate to have different physical properties from the coating in order to distinguish when the substrate has been reached.

Scanning probe methods such as AFM can also provide information regarding the topography of the surface over a given area, enabling determinations



11.3 Thin coating analysis. (a) Non-destructive techniques based upon reflected light. (b) Destructive techniques based upon probe penetration. (c) AFM surface image of an explanted polymer-coated stent where a 10 μm square of coating has been excavated by the AFM. (d) Corresponding SEM image of the excavated area. (Source: Images (c) and (d) courtesy of Biocompatibles UK Ltd, Farnham, UK, www.biocompatibles.com.)

of surface roughness and hence uniformity on the nano- as well as micro-scale. Coupled with phase analysis, the instrument can distinguish between different polymer phases or polymer and drug, as demonstrated for the styrene-isobutylene-styrene (SIBS) triblock copolymer coating on the Taxus® DES which has a microphase-separated structure.²¹ These data were complemented with transmission electron microscopy (TEM) studies, where the different polymer phases were selectively stained using RuO₄ and imaged. AFM phase imaging of the SIBS coating has been used to demonstrate the presence of the drug preferentially associated with one of the polymer phases, and to monitor the effect of drug dissolution over time on the coating morphology. This instrumental method is used to continually scan an area with increasing levels of force applied to the scanning

tip. This not only excavates an area of coating that can be used for depth profiling by cross-sectional analysis, as seen in Fig. 11.3c, but also provides an indication of the force that is required to completely remove the coating and hence a metric for investigating the influence of drug loading within the coating on its cohesive and adhesive properties. Combinations of techniques generate the most powerful data, as demonstrated by Belu *et al.* in their analysis of coatings of poly(lactic-co-glycolic acid) (PLGA) mixed with rapamycin, using surface analysis by electron spectroscopy for chemical analysis (ESCA) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) coupled with optical interferometry and confocal Raman microscopy.³⁰

More conventional mechanical property measurements can be obtained on cast polymer films (rather than the coated devices), using methods such as tensometry and dynamic mechanical analysis; but more relevant measures can be obtained using sensitive force-sensing probes that are now available (as outlined in the subsection entitled 'Physicomechanical testing'), which can be applied to the direct analysis of drug delivery coatings themselves. Nano/micro scratch testing is possible using equipment such as the Nanovea® Mechanical Tester in Scratch Test mode, which applies loads in a controlled fashion to surfaces to study failures in thin coatings. The critical load at which failure occurs is both related to test-specific parameters, such as loading rate, scratch speed, indenter tip radius and material, as well as to the test sample properties such as friction coefficient, thickness, hardness and roughness.

Part of the coating analysis needs to be conducted on the finished combination product to predict performance under clinically-relevant conditions. This type of testing can be focused on mimicking conditions for (i) the handling and administration of the product to the patients and (ii) the long-term in-use conditions over the product lifetime. The type of characterisation test selected will therefore be based around the device and how it is used in practice. Some test equipment has been designed, and is now commercially available to developers, specifically for the testing of particular devices. Bose offer a range of cardiovascular test instrumentation, including the Electroforce® 9210 DES test instrument which allows testing of 12 samples simultaneously, with pulsatile distension and particle capture technology to detect any shedding of the coating during simulated use over test periods up to ten years simulated life time.

Drug stability, dosing and uniformity determination

The drug can be present throughout the matrix of the device itself (as for an antimicrobial agent in a bone cement, or chemotherapeutic in an embolisation bead), contained within a coating on the device, or be deposited as a

layer of neat drug on the surface of the device without the inclusion of carrier (as for various DES). When combined with the device in the final product, the manufacturer must show that the drug remains stable and is unchanged over time in its combined form. This will lead to shelf-life studies on the final product format (see Section 11.5.1). The practice of matrixing and bracketing may allow a range of different drug dose and device configurations to be tested without the additional significant time and cost of analysis of every iteration in between. Again, issues arise here if one of the configurations fails and a risk-based approach would recommend archiving of certain samples for recall and detailed re-analysis in the event of an unexpected failure. Drug dose and stability can be routinely measured using combinations of high through-put UV/Visible Spectrophotometry and HPLC methods with sufficient samples to demonstrate statistical significance in dose uniformity from batch to batch.

In vitro drug-release characterisation

A vitally important property for any drug-device combination product is the rate at which the drug is released. The elution of the drug can be controlled by a wide variety of different mechanisms, ranging from simple dissolution from a surface based upon drug solubility, to controlling its diffusion through a coating or the bulk material of the device itself. Whatever the mechanism of release, there needs to be an *in vitro* method to demonstrate the controlled and reproducible elution of the drug from a combination product; this often forms part of a quality control release test. In developing such a method, a number of considerations should be made, including preparation of the device, the selection of the parameters for elution (temperature, pH, elution medium, etc.), evaluation of the sink conditions for the drug, the choice of elution apparatus, identification of the analytical method for drug detection and quantification, sampling frequency and elution discrimination studies.

The US, European and Japanese Pharmacopoeias outline a series of largely harmonised methods and apparatus for demonstration of drug dissolution and release. Although initially developed for the pharmaceutical industry to evaluate immediate release formulations, these methods have been modified to cater for sustained/delayed release modalities and for drug delivery *via* forms other than tablets, such as topical or transdermal delivery systems. These methods are also therefore being applied to the evaluation of the drug release from combination products; the principle functions of the test being:

- To allow optimisation of the therapeutic efficacy of the product (demonstrate control over timing of the dose released).

- To ensure batch to batch reproducibility of release and hence a simple measure of product quality and physical consistency.
- To allow an estimation of the *in vivo* availability of the active, often by allowing an *in vitro*–*in vivo* correlation (IVIVC) to be made (once *in vivo* data are available) whereby the test can become a predictor of product performance.
- To allow comparison of performance between different products/formulations containing the same active agents and hence an estimation of ‘bioequivalence’.

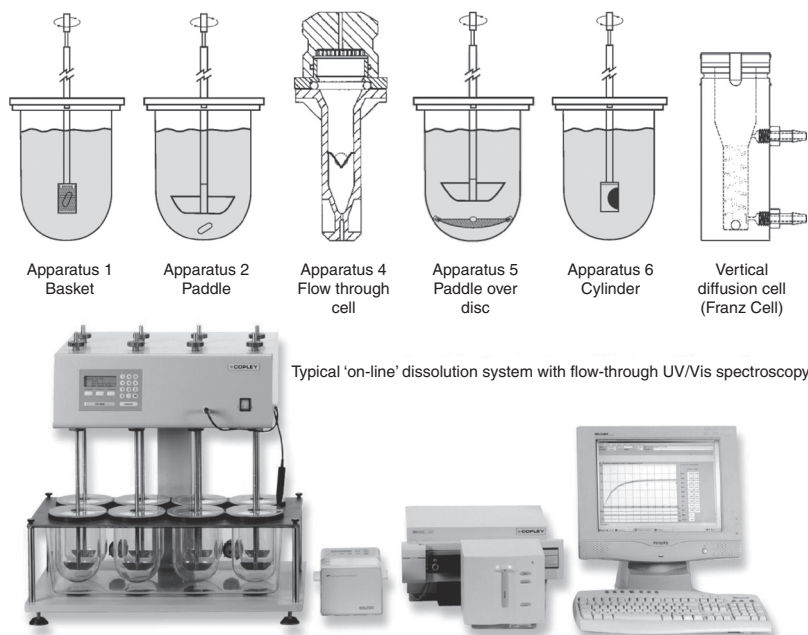
The various Pharmacopoeias therefore present suitable requirements, test methods and apparatus to defined quality standards, to ensure the safety and effectiveness of medicines. Table 11.2 outlines the many chapters from both the United States Pharmacopeia (USP) and European Pharmacopoeia (Ph.Eur.) relating to drug dissolution and release, which demonstrates the importance of this subject.

Table 11.2 Selection of US and European pharmacopoeia drug elution tests

| USP chapter | Subject | Ph.Eur. chapter | Subject |
|-------------|---|-----------------|---|
| <711> | Dissolution | 2.9.3 | Dissolution test for solid dosage forms |
| <724> | Drug release | 2.9.4 | Dissolution test for transdermal patches |
| <1058> | Analytical instrument qualification | 2.9.25 | Dissolution test for medicated chewing gum |
| <1087> | Intrinsic dissolution | 2.9.29 | Intrinsic dissolution |
| <1088> | <i>In vitro</i> and <i>in vivo</i> evaluation of dosage forms | 2.9.42 | Dissolution test for lipophilic solid dosage forms (suppositories) |
| <1090> | <i>In vivo</i> bioequivalence guidances | 2.9.43 | Apparent dissolution (powders and granules plus various monographs on dosage forms) |
| <1092> | Dissolution procedure: development and validation | | |
| <1224> | Validation of compendial procedures | | |
| <1226> | Verification of compendial procedures | | |

Apparatus for measuring drug elution

The methods and apparatus available for drug-release evaluation are selected dependent upon the dosage forms of the drug (Fig. 11.4).³¹ USP apparatuses 1–4 are concerned primarily with dissolution (mainly solid dosage forms), whereas USP apparatuses 5–7 with drug release (mainly transdermal delivery³²). The vertical diffusion cell (Franz Cell) is currently being reviewed for inclusion for the testing of semi-solid dosage forms such as creams and gels. USP apparatus 1 (basket) and USP apparatus 2 (paddle) have been used for many years in the evaluation of solid oral dosage forms and validation of such techniques is well documented.³³ The drug is released into a specified volume of elution medium at 37°C over time, which is optionally circulated through a UV/visible spectrophotometer flow-through cell that constantly monitors a selected wavelength characteristic of the drug in question. USP apparatus 2 could obviously be employed for the evaluation of a drug-device combination product, assuming the device was of such a dimension that it could be placed within the dissolution vessel and also that



11.4 Diagrammatic representation of the different USP dissolution apparatuses and a typical on-line flow-through system. (Source: Images and photograph courtesy of Copley Scientific Ltd, Nottingham, UK, www.copleyscientific.com.)

it was not damaged by the rotation of the paddle. DESs, for instance, have been evaluated with this technique; but often the amount of drug on the device is so small and the elution volume so large, that several devices are required in order to release a detectable amount of the drug. Hence, more commonly for expensive combination products such as DES is the use of one device immersed in a small volume of elution medium (typically 1–20 mL) from which aliquots are taken over time (being replaced by an equal volume of fresh medium) and then analysed off-line using a sensitive analytical method such as HPLC.²¹

Although capable of demonstrating the consistency of release of the drug from the product, these types of elution method are not representative of elution *in vivo*; here the device will be in contact with tissue into which the drug component must diffuse, as opposed to exposure to a large volume of liquid extraction medium. Hence, the relevance of the test for predicting release *in vivo* is much less. There have therefore been developments of methods that better relate to the *in vivo* situation, such as USP apparatus 4, which uses a flow-through cell into which the product can be placed and the flow of extraction medium around the product precisely controlled. Use of a USP or Ph.Eur. method is preferred where possible, as they are widely recognised and validated. Regulatory bodies, however, will accept data generated from specially-designed and validated elution tests if they attempt to measure drug release in a way more akin to the *in vivo* situation. For example, there have been reports of a modified USP apparatus 4 which attempts to make the test even more relevant, for instance by coating the interior of the cell with an alginate hydrogel into which a DES can be deployed and drug release measured into both extraction medium and hydrogel.³⁴ In the case of DEB, many have employed a so-called T-apparatus, which possesses a well into which the sample is placed and subsequently relies upon diffusion and convection processes to carry the drug into a circulating flow-through circuit where its concentration can be monitored in real-time.^{35,36} Indeed, drug-release data using this system have been subsequently correlated with human plasma drug level pharmacokinetic data, resulting in a Level A correlation, demonstrating that the technique is a good predictor of the expected plasma drug levels over the first 24 h of release from the combination product³⁷ (see the subsection '*In vitro*–*in vivo* correlation (IVIVC)').

Drug elution media

An aspect of the method employed to monitor drug elution from the combination product is the choice of medium used to extract the drug. For simple quality-control purposes, a rapid extraction medium may be required to remove all the drug from the device and allow quantification of the total dose. This may consist of a mixture of components and solvents into which

the drug is readily solubilised. For drug-release studies, it is preferred if the medium again mimics the in-use conditions as close as possible. For instance, elution into water is of limited value and as a minimum, phosphate buffered saline (PBS) would be recommended as it possesses a physiologically relevant concentration of ions. This is of particular importance where the drug-release mechanism might be dependent upon ion-exchange processes.³⁷ Determination of the solubility of the drug in standard dissolution media (as per USP chapter <724>) is therefore important, which may be known already in the literature. Where the drug is particularly insoluble in aqueous media (as for some of the drugs used in DES such as rapamycin and paclitaxel) release into PBS is too slow to be of value in an elution method. Hence, in some cases additives such as surfactants such as Tween®20,²¹ sodium dodecyl sulphate or Solutol® HS 15³⁸ are added to the elution medium to aid solubilisation of especially hydrophobic compounds. Release into more complex biological media such as plasma has been reported but there are issues with the use of this medium past a few hours as it will start to degrade.³⁷

Analysis of drug elution data

Whilst the elution experiment may give an estimate of how much and how long drug is released from the device, it is also important for determining if release is consistent from one device to the next. Discrimination between elution runs is performed by carrying out the experiment several times on the same lot of devices. Typically, dissolution data can be subjected to time-point analysis where the percentage of drug release is reported at specified time points and the values obtained analysed by conventional statistical methods such as one-way analysis of variance (ANOVA). Additionally, the US Food and Drug Administration (FDA) have provided various guidance on the application of a mathematical treatment of the elution data³⁹ that compares the profiles from the various runs and generates a number known as the similarity factor, f_2 , defined by the following equation:

$$f_2 = 50 \bullet \log \left\{ \left[1 + (1/n) \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \bullet 100 \right\}$$

where R_t and T_t are the percentage of drug dissolved at each time point. If an f_2 value between 50 and 100 is generated, the test suggests the elution profiles are identical; as the value becomes smaller, the results become more dissimilar. Different approaches to obtain the similarity factor have been reported⁴⁰ but the important objective is the use of a reproducible, robust and validated method.

11.4.2 Biocompatibility and toxicity testing

Safety testing of medical devices has been relatively well defined since the introduction of ISO 10993 and the FDA G95 memo on biocompatibility. The recent emergence of more complex combination products has seen a shift in testing strategies to include more expansive evaluation of both the device and pharmaceutical components. In the same way that biocompatibility and toxicity testing of device component material has evolved to include assessment of the final device as well, so the same is required of the combination product to account for potential interactions between drug and device. Of particular interest is the added risk posed by drug impurities, break-down products and metabolites that might be generated locally at the site of administration. It is therefore clear that a combination product requires a customised testing strategy that will take into account its component parts, their interactions, its mode of delivery and local effects of drug release, absorption and metabolism.

It is therefore not the intention of this section to go into detail on the various assays that are recommended for testing of novel device-based products. Naturally, combination products that are intended for implantation within the body should undergo, where applicable, the full range of biocompatibility tests as described throughout the rest of this book and defined under ISO 10993 (the reader is directed for instance to Chapter 15 (G. Clermont *et al.*) in reference 4 for a detailed review of pre-clinical testing for drug-device combination products). It is worth noting, however, that it may be necessary to undertake some of these tests on the device component only if the drug is known to have an influence on the outcome of the test; for example, it is pointless running a cytotoxicity assay on a DEB containing an anti-cancer agent if the drug is known to have a cytotoxic effect by a mechanism of action that will interfere with the test. A written justification for why the test has not been carried out on the combination, however, will be required for any subsequent regulatory submission.

Toxicity evaluations for combination products may involve aspects of both loco-regional and systemic effects and typically require *in vivo* studies, the duration of which will be dependent upon the end-use and expected duration of implantation within the body. This may vary from hours to months and will be determined by the biological response the test is designed to evaluate. For long-term/permanent implantable products such as DES and DEB, one, three and six month implantation studies are the norm, with possibly 12 months follow-up depending upon the expected duration of release of the drug component, its pharmacological effect and whether it already has a well-documented safety profile. Toxicity studies are acute and chronic in nature and may involve single and repeat dose evaluations. Regulatory bodies expect studies to be

performed to Good Laboratory Practice (GLP) standards, or in some exceptional circumstances to the principles of GLP if the animal model is particularly specialised (for example, certain tumour models where GLP may not be possible). This requires fully defined protocols, gross pathology of all important organs, full histopathological analysis and reporting with detailed blood chemistries where applicable.

11.4.3 Determining safety and efficacy in animal studies

The long-term *in vivo* implantations described in Section 11.4.2 and detailed more fully in Chapter 7 of this book, are designed to detect any undesired toxicity associated with the combination product that affects its ultimate safety. The objective of such studies is to provide evidence that the product can be used safely in humans and it is often required that the study be performed in such a way that a safety margin is established. For instance, the concentration of a drug component may be selected so that it is in reality several-fold higher than that selected for initial human study. In some cases, an indication of product effectiveness may be inferred from the study in addition to the demonstration of safety. For example, the porcine coronary artery model is commonly used for long-term evaluation of DES and not only provides information on the local-tissue response around the product in the vessel wall (local toxicity) but also, due to the biological response to the arterial injury, a measure on how well the drug component can prevent smooth muscle proliferation in and around the stent.^{41,42} Moreover, the model demonstrates that the device component is effective in carrying out its primary mode of action of holding open the coronary artery and maintaining good blood flow. More usually, the efficacy must be derived from an additional set of animal studies that are designed specifically to test the pharmacological activity of the drug component and/or the effectiveness of the device function.

Considerations when choosing animal models

In many cases the animal model will not be able to provide particularly relevant information regarding the efficacy of the product and indeed, even in those models designed to test effectiveness, the translation from animal to human pathophysiology is usually poor and provides only a weak indication that the product could work. Conversely, if the active under study has particular specificity for human biology, the inappropriate selection of an incompatible animal model to demonstrate efficacy could prematurely end the product development of a potentially effective therapy. Relevance of the model to the human condition is therefore an important consideration.

Case studies: drug-eluting intra-arterial devices. As mentioned in Section 11.4.3, the porcine coronary artery model is commonly employed in the evaluation of DES; the biological response induced by the balloon injury in young pigs, however, is only a surrogate at best for the complex and varied coronary artery disease (CAD) state manifested in largely elderly and diabetic patients. Other models, such as the use of mini-pigs, allow longer-term follow-up of the animals post-implantation, as standard farm swine grow too large to manipulate easily within theatre; but these still do not possess CAD representative of humans. Some specialist animals have been bred specifically for such studies; hypercholesterolaemic rabbits manifest CAD where there is more biological similarity in the disease processes with that of the human condition.⁴³ These animals however, have smaller arteries and device placement in the heart is not usually possible; implants in the larger aortic or iliac arteries thus have the disadvantage that they do not have the organ-specific environment.

A similar situation exists for DEBs – a product designed for intra-arterial delivery into the hepatic artery to block blood flow to liver tumours and concomitant delivery of a chemotherapeutic agent. A relatively simple model of hepatic arterial embolisation is sufficient to demonstrate safety of the product^{17,44}, enabling detailed pathological analysis of the effects of the combined arterial occlusion and local drug delivery, in addition to the ability to evaluate pharmacokinetics and hence estimate gross systemic exposure to the drug. This model does not, however, provide any indication of whether the therapy would be suitable for treating a vascular solid tumour. A tumour model, preferably of hepatic origin or at least hypervascular in nature, is therefore desirable to demonstrate relevant efficacy for this product. Large animals such as sheep and pigs possess relevant arterial dimensions but there are currently no large animal liver tumour models. Again, the rabbit provides one approach with the well-characterised VX-2 tumour model, which is hypervascular in nature and can be treated with a microcatheter and intra-arterial administration of the product. A tumour-bearing rat model of colorectal cancer to the liver has also been used in an embolisation setting.^{45,46} This model, however, requires a complex surgical approach to allow infusion of the beads directly into the hepatic artery, as access using microcatheters is impossible. Furthermore, the use of this model depends upon a specially-made product that is small enough to pass through the tiny arteries of the rat and it must be recognised that the drug-release kinetics of such a product could differ significantly from that being developed for human use. Therefore, as with all pre-clinical studies, caution must be taken when translating the results to the clinical setting.

Dose-ranging/finding studies

Dose-ranging/finding studies may be necessary to determine the appropriate dose that demonstrates both safety and efficacy. It may therefore be necessary to perform studies in a number of different animal models, as it may not be possible to obtain both safety and efficacy data in the same model (see the subsection entitled 'Considerations when choosing animal models'). Additionally, it is usually a requirement that tests are performed in more than one animal species. Such studies may involve both acute and repeat administration of the product where possible with subsequent assessment of the resulting toxicity. The aim is to determine the NOAEL (no observed adverse effect level) for the drug component and will mimic the dosing and frequency of administration that would be proposed for the clinical use of the product. Often it is desirable to demonstrate a safety factor to allow for interspecies differences such as tolerance to the drug or rate of its metabolism. An appropriate safety margin may be determined based upon milligrams of drug per unit mass or surface area per day in order to translate to a human equivalent dose. These types of study are best carried out to GLP standards under approved protocols in recognised models where applicable. Fully documented autopsy reports are required with gross and histopathological examination of the relevant tissues/organs.

Pharmacokinetics and bioavailability

If the drug component is well known and understood, it may not be necessary to conduct extensive absorption, distribution, metabolism and excretion (ADME) studies. By delivering a drug from a device, however, it may be that the extent and duration of exposure to the active agent is altered compared to its normal route of administration. Drug pharmacokinetics (PK) therefore become an important aspect for the combination product and one of the key challenges in generating these data is often the relatively low doses of drug that are delivered. This would normally involve taking tissue local to the implant site, specific target organs and/or plasma samples, at various time points over a relevant period. The drug is extracted from the tissue (which in itself may require significant method development for the efficient removal of all of the drug) and then subjected to analysis using a sensitive analytical method such as HPLC or mass spectrometry. It may also be necessary to monitor the main known metabolites of the drug to gain an insight into whether there is a change in how the drug is processed within the body as a result of the change in delivery route. Depending upon the type of device under investigation, it may sometimes be difficult to separate the device from the tissue and hence the extraction process will remove drug that is both resident in the tissue and in the device itself. Even then, levels of the drug may be so small that other techniques such as radiolabelling

or those that focus on the higher concentrations usually found local to the implant site may be employed (see the subsection on 'Local drug distribution'). It may be possible to correlate the PK data by coupling it with the *in vitro* drug-release data obtained as outlined in the subsection 'In vitro drug-release characterisation' to obtain an IVIVC, which is the subject of the following section.

In vitro–in vivo correlation (IVIVC)

IVIVC is defined as the relationship between *in vitro* dissolution and *in vivo* input rate. In many cases, however, dissolution is not the rate-limiting step in the elution of a drug. The FDA guidance on IVIVC provides general methods for establishment of IVIVC for oral formulations, but there is very little information available on the development of appropriate *in vitro* methods for IVIVC for non-oral forms.³⁹ The principles of this guidance can be applied to develop IVIVC for non-oral formulations⁴⁷ and although there have been a number of reports on *in vitro* methods correlation with non-oral drug-release formulations, no standard procedure has been adopted.^{48–50} In cases where a meaningful IVIVC can be developed, it can be used to predict the biological performance of a dosage *in vivo*. This may minimise the number of *in vivo* studies required for dosage form development, allowing prediction of potentially toxic or ineffective formulations with both cost and ethical benefits.⁵¹

The IVIVC can be of three types:

- *Level A correlation:* Generally linear (although not always) with a point-to-point relationship between *in vitro* dissolution and some parameter derived from the *in vivo* data. Level A is the most useful and valuable type of correlation model and recommended where possible.
- *Level B correlation:* Uses statistical moment analysis, which compares mean *in vitro* dissolution time with mean drug residence time or mean *in vivo* dissolution time.
- *Level C correlation:* This establishes a single point relationship between a particular pharmacokinetic parameter (such as C_{\max} or AUC) and a dissolution/elution parameter (such as the time to reach x% dissolution/elution, or a dissolution/elution rate). This is considered the weakest of the correlations but may often be described in the context of multiple Level C correlations for a particular system.

A Level A correlation will aid in accelerating some aspects of the combination product-development cycle by providing a predictive tool that links the *in vitro* release characteristics with the *in vivo* performance. In order to maximise the chances of obtaining a Level A correlation, time should be

spent obtaining the optimal *in vitro* release data, with special attention to the relevance of the release apparatus, conditions and elution media (see the subsection on '*In vitro* drug-release characterisation').

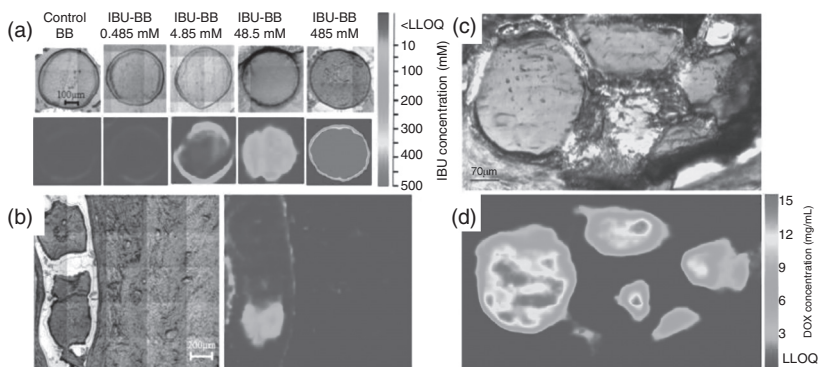
Local drug distribution

Obtaining information on the local drug distribution in tissue delivered from a combination device is one of the major analytical challenges faced by the developers of the products. The difficulty in obtaining such data is somewhat related to the properties of the drug itself, in as much as how difficult it is to detect in low quantities. Naturally it is possible to adopt the methods used in the pharmaceutical industry when addressing questions such as biodistribution and fate of an entity. In such cases radiolabelling studies are commonly employed but these are invariably expensive in nature, as the drug has to be synthesised with a suitable non-labile radionuclide and the experiments carried out in facilities capable of handling radioactive compounds. Nevertheless, this technique remains a useful method for the analysis of combination products and one of the few that will enable an account of the whereabouts and fate of all of the drug post-delivery. Radiolabelled angiopeptin has been delivered from DES and autoradiographical images of histological sections generated to demonstrate that the drug diffuses from the stent coating and into the vessel wall;⁵² moreover it showed the drug was still found in the surrounding tissue at least 28 days later.⁵³

Where the drug is more easily detected, optical or spectrophotometric methods can be applied. DEB containing the highly fluorescent chemotherapeutic agent doxorubicin have been used in both animal and human studies where the tissue has been later recovered for analysis the drug distribution.⁵⁴ Standard epifluorescence microscopy can generate spectacular images of drug penetration through the vessel wall and into the surrounding tissue over time.⁵⁵ Microspectrofluorimetry has been applied to histological sections of the processed tissues and, by comparison with a series of controls derived from collagen imbedded phantoms, quantification of the drug levels in the tissue as a function of distance from the implant surface were obtained. Similar studies have also been performed using infrared microspectroscopy, which is useful where the drug is not inherently fluorescent and hence more difficult to detect.⁵⁶ Coupled with the imaging capability of this technique it has been possible to generate images of drug distribution within, and quantification of *in vivo* release from, drug-eluting implants containing drugs such as doxorubicin, irinotecan and ibuprofen (Fig. 11.5).

Antimicrobial efficacy (AME) testing

One area of combination product development that is becoming increasingly common is that of temporary or permanent device implants which



11.5 (a) Upper panel, bead block (BB) containing ibuprofen (IBU) of different loadings, embedded and sectioned; lower panel, corresponding FTIR image at 1512 cm⁻¹ to visualise drug content in the beads. (b) Left panel shows histological section of IBU-BB in a uterine vessel; right panel shows corresponding FTIR image at 1512 cm⁻¹ showing IBU distribution. (c) Histological section of a vessel occluded with DEB containing doxorubicin (DOX); (d) corresponding FTIR image at 988 cm⁻¹ showing distribution of DOX. (Source: Images courtesy of Dr Julien Namur, Archimmed SARL, France.)

deliver antimicrobial agents to tackle infections at the implant site. Such infections are not uncommon (typically 1–5%) and can be notoriously difficult to treat with systemic therapies. Often the only course of action is device removal, which can be risky and is a considerable extra expense. By releasing a suitable antimicrobial agent at a therapeutic dose and over an appropriate time scale, any infection introduced at the time of the procedure or subsequently during wound-healing may be controlled more efficiently. It is therefore necessary during the development of such combination products, not only to have an understanding of the safety and pharmacokinetics but also to have evidence that the antimicrobial agent that has been selected is efficacious against the pathogens likely to be the cause of any potential infection. Table 11.3 outlines a selection of available methods suitable for the *in vitro* testing of antimicrobial combination products.

Effectiveness is typically measured by inoculation of the test product with known levels of a test organism (dependent upon the most common cause of the infection targeted by the product, frequently *Escherichia coli* and *Staphylococcus aureus*). The reduction in microbe count is then measured over a 1 day (ISO 22196) to 28 day period (USP<51>). ASTM E-2180 is a specific test for antimicrobial agents contained within hydrophobic polymer materials (which may be the device itself or a coating material), which

Table 11.3 Selection of AME test methods relevant to combination products

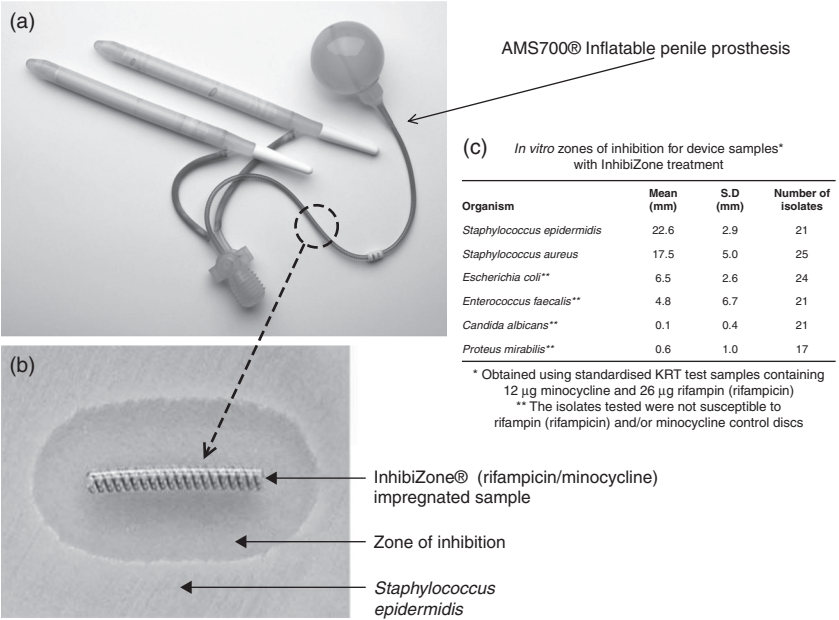
| ASTM | Test |
|-------------|--|
| USP <51> | Antimicrobial effectiveness |
| USP <1227> | Validation of microbial recovery (neutralisation validation) |
| ISO 22196 | Antimicrobial activity, quantitative |
| ASTM E-2149 | Dynamic contact (antibacterial, quantitative) |
| ASTM E-2180 | Bound antibacterial activity |
| ASTM E-2315 | Time-kill procedure |
| ASTM G-21 | Antifungal (semi-quantitative) |
| Other | Zone of inhibition test |

is contacted with a solution of a challenge organism such as *Pseudomonas aeruginosa* to mimic biofilm formation on the device. Percent reductions in the organism over time are calculated comparing the active test article versus a non-active control. ASTM E-2315 is applied when the product is intended to act over a prolonged time period and a kill time-course model is required. Zone of inhibition (or Kirby-Bauer disk diffusion assays) also provides a visual basis of measuring the potency of an antimicrobial that is released from a material by creating an area of no growth around the test article. This is illustrated in Fig. 11.6, with the AMS700® inflatable penile prosthesis. This device is impregnated with the antibacterial compounds rifampicin and minocycline, which clearly demonstrates an active zone of inhibition against organisms such as *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Having demonstrated *in vitro* antimicrobial capability, regulatory bodies will usually also require an efficacy measure for the product in an *in vivo* infection model. Here, the devices can be implanted into subcutaneous pockets in a suitable animal, typically a rabbit, which is infused with a clinically-relevant strain of microorganism. After a specified time (which can be days to weeks), the implants are retrieved aseptically and the remaining viable organism quantified by various techniques. This testing can be accompanied by other evaluation such as histological assessment or analysis of local-tissue levels of the antimicrobial agent. All of this would be compared to non-active positive control implants.

11.4.4 Common inadequacies in pre-clinical testing

The testing outlined in this section should be performed in a thorough manner and to high quality standards for inclusion in regulatory submissions. Animal studies should always be performed to GLP. On rare occasions where GLP may not be possible, for instance in the use of a highly-specialised experimental model, it may be acceptable to argue exclusion of certain



11.6 (a) AMS700® Inflatable penile prosthesis. (b) Zone of inhibition experiment demonstrating antimicrobial efficacy of a section of tubing against *Staphylococcus epidermidis*. (c) Table of zone of inhibition data from the device against various organisms. (Source: Photographs courtesy of American Medical Systems, Minnetonka, Minnesota, www.AmericanMedicalSystems.com.)

subparts of the study from the GLP certificate. Nevertheless, the highest quality standards are expected by regulatory authorities and lack of appropriate traceability and documentation for such studies is a common cause of submission failure. The complexity involved in pre-testing of combination products therefore often results in inadequacies in the test data submitted for review. Portnoy and Koepke provided a relatively recent review of the most common inadequacies encountered in submissions,⁷ which have been reproduced in Table 11.4 as an illustration to the developers of such products where potential pitfalls may lie.

11.5 Aspects to consider in the manufacture of combination products

The combination product will be subjected to the detailed *in vitro* and *in vivo* characterisation outlined in the previous sections throughout its development phase in order to optimise its performance. These types of tests are

Table 11.4 Common pre-clinical testing inadequacies in combination product submissions

| Pre-clinical test | Common inadequacy |
|-----------------------|---|
| Bench evaluation | <ul style="list-style-type: none"> • Inadequate fatigue and corrosion testing • Inadequate analysis of surface modifications |
| Laboratory evaluation | <ul style="list-style-type: none"> • Inadequate testing of drug-coating integrity and durability |
| CMC evaluation | <ul style="list-style-type: none"> • Inadequate particulate analysis (USP <788>) • Inadequate demonstration of chemical stability • Inadequate characterisation of drug content and uniformity • Incomplete <i>in vitro</i> pharmacokinetic testing • Poorly characterised CMC methodologies and specifications • Inadequate characterisation of impurities • Unacceptable or poorly characterised toxicity data for leachables and/or residual solvents • Insufficient data on chemical effects of sterilisation on finished product • Inadequate demonstration of product stability/shelf-life |
| Animal studies | <ul style="list-style-type: none"> • Insufficient data to provide preliminary evidence of safety • Inadequate evaluation of clinically intended dose • Inadequate evaluation of overdosage • Unacceptably short-term duration of chronic follow-up • Inadequate evaluation of local-tissue toxicity • Inadequate evaluation of systemic toxicity • Inadequate or missing description of histopathology • Necropsy reports not included in submission (especially important for unexpected animal deaths) |
| Clinical evaluation | <ul style="list-style-type: none"> • Issues involving the duality, duration, and/or applicability of feasibility data • Omission of dose-ranging studies • Failure to fully consider pharmacological aspect of product • Failure to provide complete and comprehensive clinical results |

Note: CMC = chemistry, manufacturing and controls.

Source: Reprinted with permission from *Medical Device + Diagnostic Industry*, 'Regulatory Strategy: Preclinical Testing of Combination Products', May 2005 (ref [7]) Copyright © 2005 Canon Communications LLC.

best carried out when the design of the product has been frozen and there are no other planned changes to the components, such as in concentrations of actives or carriers, inclusion of excipients, or alterations to the processes used in fabrication that could introduce new or increased levels of residuals. Such changes could affect the performance characteristics of the product and ultimately its safety and effectiveness profile. If changes are required

in order to facilitate scale-up to manufacturing capacity, additional bridging studies may be required to show the combination product performance remains unchanged.

11.5.1 Shelf-life

Shelf-life is an important parameter that should be considered at an early stage. The stability of the final combined product defines how long it can be held in inventory or on the customer's shelf from the date of manufacture before it exceeds its shelf-life and must be discarded. Shelf-life testing can be performed under accelerated conditions for a quicker indication of stability; often this is not the recommended approach, as the components of the product may be more susceptible to degradation at the elevated temperatures used in the test and regulatory authorities do eventually require real-time ageing data under ambient conditions. The product should be tested against its release specification to ensure it continues to perform as predicted; this may involve a range of different tests, often composed of those described throughout this chapter that have been used in the characterisation of the product throughout its development. A typical measure for a drug component would involve extracting the drug from the product by a validated method and subjecting it to a sensitive analytical technique such as HPLC to ensure the drug has not undergone any degradation. The kinetics of drug release should also remain unchanged and will need to be tested to a pre-determined percentage of total dose (normally at least 80% elution) and compared to the reference in the product specification using the similarity method outlined in the subsection entitled 'Analysis of drug elution data'. As with shelf-life, shipping studies also require the same type of evaluation of the product after it has been sent to and back from a specified destination in order to simulate transport conditions. Typically, humidity and temperature would be monitored during shipping and attention would also be paid to the integrity of the product packaging.

11.5.2 Sterilisation

Combination products are usually supplied sterile and may be sterilised by conventional terminal sterilisation techniques such as ethylene oxide (EtO), steam sterilisation or gamma irradiation. It may be that certain components of the product could be destroyed by the sterilisation step and it is necessary to undertake the required level of characterisation testing to demonstrate that the performance characteristics of the product remain unchanged post-sterilisation. Sterilisation by EtO is often a batch process and it must be shown that there is no cross-contamination from the residuals of a previous

sterilisation run or different product processes being undertaken at the same time. Again, analytical methods, such as HPLC coupled with techniques that provide structural information, such as nuclear magnetic resonance (NMR) spectroscopy can be useful in determining if a drug is altered by the sterilisation process. If this turns out to be the case, the method of manufacture may have to be altered in order to produce the combination product under aseptic conditions, which requires an additional level of control and testing to ensure sterility is maintained in the end product.

11.6 Clinical studies for combination products

Whilst combination products may have great potential to deliver significant clinical benefit to patients, the use of highly innovative cutting-edge technologies could in itself raise complex scientific questions. This may require the development of novel methodologies for the evaluation of the product performance, as we have seen in the proceeding sections of this chapter. Clinical trial design to establish safety and efficacy of a combination product is no exception to this; particularly given the involvement of the different regulatory paradigms under which these products are usually developed.

11.6.1 Combination product retrieval studies

Although now somewhat beyond the scope of this chapter, the pre-clinical data generated in support of the safety and efficacy of a combination product will be used in a submission to approve clinical evaluation of the investigational product. Many of the techniques and methods developed and applied at the pre-clinical stage may also be of utility in the clinical phase; for instance, methods developed for PK analyses, or techniques for evaluating the product if it becomes available post-explant of the device, or tissue in which it is sited. For example, the AFM technique described in the subsection ‘Evaluation of drug delivery coatings’ was developed and used to determine the thickness of a stent coating, both post-processing and also after *in vivo* implantation. During a routine clinical procedure on a patient, a coated stent was captured by accident and a section of it retrieved for analysis.²⁸ The same AFM techniques were applied to demonstrate that the coating remained intact and maintained its original thickness and mechanical properties, which was a useful validation of the pre-clinical data.

In some cases it is possible for the removal of the product to be planned as part of specific treatment regime. DEB loaded with doxorubicin were used in a study to treat patients with hepatocellular carcinoma (HCC), in order to keep their disease under control whilst they were on a liver transplant list. Once a donor liver became available, the patient had the transplant and

the diseased liver (containing the product) was subsequently analysed in order to elucidate the whereabouts of the beads and the distribution of the drug in the tissue. A number of patients were treated with bridging periods from just 1 day to 36 days, which allowed a time-course of drug distribution within the liver to be recorded. Once again, this study validated the pre-clinical findings, which demonstrated that the drug levels and time-course generated in a non-tumour-bearing porcine liver embolisation model were very similar to those observed in human patients with HCC.⁵⁷

11.6.2 Brief consideration for combination product clinical trial design

Clinical evaluation of a combination product needs to be designed to determine its performance, not only in terms of safety, but also that there is a clinical benefit that arises as a consequence of the combination. The degree of clinical evidence required will probably depend upon the nature of the product and the label claims being pursued. When planning a clinical trial, a cost-benefit assessment must therefore be made to determine the financial viability of conducting such a study. For example, if the combination product contains an antibacterial compound and the manufacturer wishes to claim reduced rates of infection, yet the infection rate for the standard device is already relatively low, the patient numbers required to demonstrate a statistically significant decrease in infection rate in a randomised controlled study are likely to be so high as to make such an investment untenable. Hence, the manufacturer may wish to consider an alternative indication to the efficacy claims, which may be attainable based upon pre-clinical data alone.

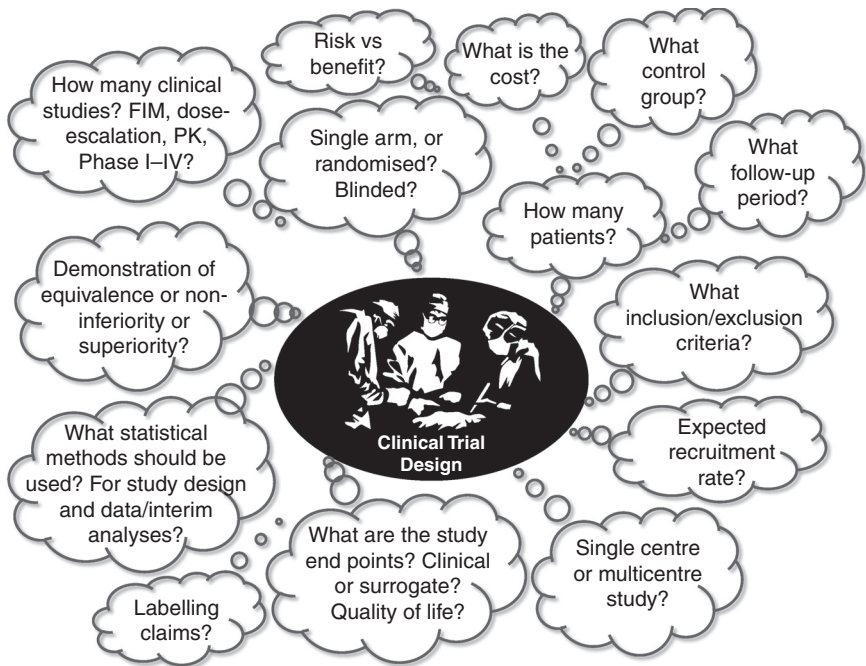
Unlike clinical studies for medical devices, the combination product may require several phased clinical trials similar to those conducted for medicinal products. This may involve a dose-escalation aspect to determine or evaluate a maximum (tolerated) dose, as was the case for the first studies of DEBs used in the treatment of liver cancer.^{58,59} In these phase I/II type studies, a first cohort was treated with a fixed volume of beads, in which the drug dose was escalated over groups of three patients to a maximum dose (based upon the current maximum recommended systemic dose). Once it was ascertained that this dose was safe with no dose-limiting toxicities, the second cohort were then treated with this maximum dose. This study also involved a pharmacokinetic analysis to determine systemic drug levels released from the device, which could be later compared with the pharmacokinetic data generated from pre-clinical models and correlated with *in vitro* drug-release data.³⁷ Similarly, for many of the first-in-man DES studies, a number of trials were performed with either different drug doses on the stent,⁶⁰ or different release modalities (e.g. fast, moderate or slow release

formulations),^{61,62} reflecting that the period over which the drug is eluted from the device will be critical depending upon the underlying biological response that is being addressed.

These types of ranging studies are then usually followed-up with bigger studies under intended-use conditions.⁶³ An approval for a combination product will require a randomised study that proves statistically that the product is at least equivalent or better (by whatever metric is deemed central to the function of the product) compared to the existing standard of care; these sorts of trials are usually multicentre in nature to achieve the necessary recruitment of the hundreds of patients that will be required to drive a positive statistical outcome that is adequately powered. The applicant and regulatory body will negotiate/discuss the details of the clinical trial design that is required in order to support the marketing authorisation application for the product. Active control arms are preferred to those based upon a placebo, not least for ethical reasons. The advantage of a combination product that is the first of its type is that it may be randomised against current therapy as a control where it may require fewer patients to power a delta in performance. Early studies for DES randomised against bare metal stents and easily demonstrated superiority;⁶⁴ nowadays, new DES designs are compared against currently approved products and trials geared towards proving substantial equivalence in performance.⁶⁵ The intention of this section was to provide a flavour of what is involved in clinical evaluation of combination products and to demonstrate that it is a complex matter that encompasses a wide variety of considerations (see Fig. 11.7); however, the details of these considerations are well beyond the scope of this chapter.

11.7 Conclusion and future trends

Combination products offer potential for a step-change in product performance and subsequently significant clinical benefits to the patient. As more novel combination products are being developed and brought to market, there must also be the development of characterisation methods that are both appropriately designed to reflect the function of the product and also analytically sensitive enough to detect the low levels of active compounds present. Truly representative *in vivo* models that provide valuable information on both safety and effectiveness aspects of the combination product remain one of the biggest challenges. Regulatory bodies are experiencing an increase in combination product submissions and are responding by creating departments and guidelines to help manufacturers through the approval process. However, the presence of both device and drug elements is, and will continue to cause some confusion and debate, as those offices responsible for reviewing these elements approach product approvals from different perspectives. The successful product developers will be those that



11.7 Key considerations when designing a clinical trial programme for a combination product.

engage with the regulators at an early stage to define the requirements for an approval, enabling them to design and employ the most suitable characterisation methods in order to demonstrate their product is ready for human use.

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Methods and interpretation of performance studies for bone implants

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Abstract: Evaluating performance of implants at an early stage, long before the clinical introduction to the market, has proved to be challenging. Researchers, regulatory affairs organizations and authorities are looking for models capable of eliminating poorly performing products in order to select the right candidates for further development.

This chapter was designed to provide help in the selection and interpretation of appropriate methods in order to assist the development of new bone implants.

Key words: bone implants, performance, non-clinical studies, bone repair, biodegradable, osteogenesis, osteoconduction, sensitivity, method.

12.1 Introduction

Human patients of various ages are awaiting new therapies to improve their conditions following traumatic, tumoral, infectious, developmental and inflammatory diseases.

New bone therapies raise fewer issues of safety than of performance. Unfortunately, many implantable products are still developed and used clinically over long periods of years, until it is established that their performance is not satisfactory and the products are abandoned.

Evaluating performance of these products at an early stage, long before the clinical introduction of such products, has proved to be challenging because of the difficulties related to the proper selection of non-clinical performance models. Researchers, regulatory affairs organizations, and authorities are looking for non-clinical models capable of eliminating poorly performing products as early as possible in order to select the right candidates for further development.

This chapter is designed to provide concise and practical help in the selection and interpretation of appropriate methods in order to assist the development of new bone implants.

12.2 Definitions

- **Performance:** the accomplishment of a given task measured against pre-set known standards of accuracy, completeness, cost, and speed. More complete information on the definition of implant performance can be found in Chapters 14, *Non-clinical functional evaluation of medical devices: general recommendations and examples for soft tissue implants* (Dr Gaelle Clermont), and 13, *Methods and interpretation of performance studies for dental implants* (Dr Michel Dard).
- **Bone implant:** a medical device inserted or grafted into bone for prosthetic, therapeutic, diagnostic, or experimental purposes.
- **Bone regeneration:** the formation of bone with histological, biochemical, and mechanical properties similar to that of native bone.¹
- **Non-clinical studies:** studies designed to collect information from *in vitro* or *in vivo* test systems. More information is provided in Chapter 13.
- **Bone repair:** the process of healing injured bone through cell proliferation and synthesis of new extracellular matrix.¹
- **Endochondral ossification:** one of the two main types of bone formation, where a cartilaginous matrix forms first and is subsequently replaced by osseous tissue.
- **Remodeling:** a lifelong process where old bone is removed from the skeleton (bone resorption) and new bone is added (bone formation).
- **Skeletal maturity:** the age at which the growth plates are fused.
- **Critical-size defect:** the smallest sized intraosseous defect, either naturally occurring or artificially created, in a particular bone and species of animal that will not heal spontaneously during the life time of the animal or that will show less than 10% bone regeneration during the life time of the animal.^{1,2} In the clinical setting, this term applies to defects exceeding a healing period of approximately 6 months in otherwise healthy adults.
- **Bioabsorbable:** capable of being absorbed, whether metabolized or not, and eliminated by tissues and organs.
- **Biodegradable:** capable of being broken down (or decomposed) into smaller fragments or molecules through the action of cells, enzymes, dissolution or mechanical processes. Biodegradation is typically the first phase in the process of bioabsorption; however, some materials are biodegradable but not bioabsorbable, resulting in storage within tissues or organs.
- **Negative control product:** a product that, under standardized conditions, confirms the capacity of the selected model to provide a reproducible negative response. With regard to performance studies, a negative response is considered to be an unsatisfactory response. An

unsatisfactory performance with regard to implant osteointegration is illustrated by [Plate IX](#) (see colour section between pages 246 and 247).

- **Positive control product:** a product that, under standardized conditions, confirms the capacity of the selected model to provide a reproducible positive response. With regard to performance studies, a positive response is considered a satisfactory response. A satisfactory performance with regard to implant osteointegration is illustrated by [Plate IX](#).
- **Reference product:** in this chapter, a reference product is defined as a product that under standardized conditions confirms the capacity of the selected model to provide a reproducible known *positive* response. A reference product is typically a well-established product, registered for a target market for a minimum of 2 years, not associated with adverse materio-vigilance events induced by the product itself. The reaction induced by a reference implant is shown in [Plate IX](#).
- **Test material, test article, test implant:** the product, product part or component selected to be studied for the purpose of non-clinical performance and/or safety assessment.
- **Osteogenesis:** the process by which osteogenic stem cells and progenitors create new bone tissue through processes of homing, activation, proliferation, migration, differentiation and survival.³
- **Osteoconduction:** a scaffold function that promotes the attachment, migration, distribution and survival of osteogenic cells within a defined tissue volume and/or within the surrounding area of an implant.³ Bone ingrowth by osteoconduction onto a hydroxyapatite layer is illustrated in [Plate X](#) (see colour section between pages 246 and 247).
- **Osteoinduction:** traditionally defined as the process by which soluble or matrix-bound signals interact with local cells (progenitors or non-progenitors) to initiate a cascade of cellular events that changes the fate of local uncommitted stem cells or progenitors toward an osteoblastic phenotype.³
- **Mass transport:** general process of flux and exchange of chemical modalities through tissue by convection (physical and mass movement along pressure gradients, as in vascular channels or in extracellular fluid spaces exposed to deformation via movement or external deformation), diffusion (passive migration of chemical species along concentration gradients of a submicron to millimeter scale) or active pumps (usually acting only across membranes at short distances).³
- **Reproducibility:** the capacity of a model to provide the same or similar results in repeated experiments in the same laboratory and in identical experiments in different laboratories.³

- **Sensitivity:** the capacity of a model to enable detectable differences in outcome based on change in a particular test variable, preferably the nature of the test implant.³
- **Specificity:** the capacity of a model to provide an experimental design that isolates a single independent variable for assessment.³

12.3 Scope

The scope of this chapter is:

1. To summarize information and principles to guide investigators in the choice of reliable models to evaluate innovative bone implants.
2. To provide information on the current *in vivo* models used for regulatory approval in the United States, Europe, Japan, China and the rest of the world.
3. To provide data that may help interpret results and reach appropriate conclusions from non-clinical performance models.

Innovative bone therapies are not limited to bone implants; however this chapter will focus on models that are commonly used to assess bone implants based on materials or on an association of materials with drugs or cells. Models that are based upon the use of pure drugs and biologics will not be reported. Non-clinical performance models used to assess cell therapy strategies specifically have been described precisely in guidelines published by the US Food and Drug Administration (FDA).⁴ The reader interested in learning more about combination products associating a drug or a biologic with a medical device may refer to Chapter 11 *Methods for the characterisation and evaluation of drug-device combination products* (Dr Andrew Lewis) in this book or may refer to the book edited by Dr Andrew Lewis.⁵

The design of performance studies varies based upon the developmental stage of the product: fundamental discovery models are not considered in this chapter, whereas proof of concept performance models and models used to confirm performance at the end of the non-clinical developmental stage for a regulatory approval are included.

Ethical principles and animal welfare requirements are described separately.⁶ Chapters 15, *Mechanical testing for soft and hard tissue implants* (Dr Christian Kaddick), and 19, *Microscopic and ultrastructural pathology in medical devices* (Drs Antoine Alves, Alan Metz and J. Render), in this book provide more specific information on mechanical and histological methods used to evaluate bone implant performance.

Finally, details concerning *in vivo* models used to evaluate the performance of orthopedic implants other than bone implants, such as ligament or

tendon repair and fixation devices, meniscal, articular cartilage and articular joint devices can be found in the book by Drs An and Friedman.⁷

12.4 Principles for the selection of an *in vivo* model to evaluate performance of bone implants

The selection of an *in vivo* model is guided by the bone repair processes and anatomical differences, category of implants, study objectives and model-specific characteristics.

12.4.1 General principles and examples

The nature of the bone-repair processes within a bone implant or in the surrounding area are roughly similar in nature in various animal species and in man. Implantation is followed by a local inflammatory reaction.⁸ Similar to the intramembranous ossification process in the developmental phases, the osteoblastic pathway leads directly to new bone formation while the chondroblastic pathway generates new bone via endochondral ossification. Generally, both processes coexist in normal bone repair. However, one pathway may quantitatively predominate in certain species. For example, endochondral bone formation predominates in rodents.⁹

Quantitatively, species variations and anatomical location-based variations are observed in regard to bone repair performance.^{10–13} For example, bone repair occurs faster in the cranio-maxillo-facial area than in the diaphysis of long bones.

In most cases, the selection of a model will not be based on differences in the nature of the repair process, such as the bone repair pathway that predominates in a particular species, but on considerations based on size. This is illustrated by the two following examples.

Consider a study whose purpose is to establish an early proof of concept regarding the osteoconductivity potential of a test implant, with secondary endpoints focused on the kinetics of degradation and signs of local inflammatory reaction. In this case, the selected model is often a small cylindrical defect with a diameter in the range of 1–6 mm in the metaphyseal femur of rodents or rabbits. However, if a porous scaffold material designed to release a growth factor is under investigation, mass transport performance is the primary endpoint. Hence, the capacity of the porous scaffold to induce new blood vessels and other tissues in growth should be evaluated, despite profound hypoxia within a large sized bone defect. In this case, the situation requires a larger defect in the range of 10–15 mm diameter maximum and should be tested in a large animal such as the sheep or the goat.³

12.4.2 Proof of concept studies

Proof of concept studies can be performed in rodents such as mice, rats, or in rabbits. Such studies may sometimes be conducted in a small number of large animals. In this latter case, however, any differences observed between treatments cannot be analyzed statistically, but only submitted to a biological interpretation, by reason of the low number of specimens. Consequently, any lack of difference observed may be interpreted as either an absence of difference regarding the performance of the test implants or as a result of the limited size of the study group. Conducting a proof of concept study with large animal models is advisable in the following situations: when the surgical procedures cannot technically be performed in a smaller animal, when the size of the implant exceeds the volume capacity of the species or when there is a need for a model of the condition of a large bone defect.³

12.4.3 Can a single model fulfill all requirements?

Models can be selected by giving careful consideration to the risk of natural variation observed between animals. For example, while a study with small standardized bone defects in small rodents or rabbits of uniform ages and genetic backgrounds limits animal-to-animal variation, it may not mimic the clinical conditions of use of the implant. On the other hand, a study of a segmental (e.g., long bone diaphyseal) critical-size defect in a large animal may not fully satisfy the requirements for age and genetic uniformity. However it may better mimic the clinical situation in terms of mass transport and environment (e.g., oxygen diffusion, concentration and gradient of signaling molecules or vascularization rate and progenitor cell migration and concentration) as well as mechanical constraints, which are highly dependent on defect size.^{14–16} As these examples show, it is generally recognized that no single model can fulfill all requirements.

12.4.4 Criteria used to select a model

The review of publications in six prominent orthopedic journals over ten years showed that rodents were predominantly used to study fracture repair (53%) followed by rabbits (19%), sheep (11%), dogs (9%) and goats (4%).¹⁷ The representation of smaller species was high because early stage research and fundamental research studies were included in the review. However, regulatory performance studies are conducted mostly in large skeletally mature animals rather than in rodents or rabbits. In addition, the percentage of studies involving sheep or goats in orthopedic research increased by 50% during the period between 1990 and 2001 compared with 1980–1989,

and by another 50% during the years 2001–2009.¹⁸ Rabbits may still be used in selected cases to improve standardization and obtain more uniformity in group comparisons.

The use of large animals permits one to mimic fractures, osteotomies, and defects. Dogs, goats, and sheep are commonly used because they are more likely to provide results like those that would be seen in human clinical studies. Again, rodents may be used, but are not recommended due to differences in bone remodeling: in man and large animal species, Haversian remodeling occurs by tunneling osteoclasts, whereas this does not happen in rodents. Continuous growth-plate activity is also specifically observed in rodents. Rodents heal faster than larger animals and humans.¹⁹ For example, full fracture repair of a large size tibial defect is expected to occur before 12 weeks in the rat whereas it requires 20 weeks in the rabbit ulna and radius or even 32–52 weeks in the goat or sheep tibia.²⁰

Species related differences

The osteonal secondary bone structure characteristic of the human anatomy is limited in the dog to the core of the cortical bones, and laminar bone is observed mainly in the areas close to the periosteum or endosteum. Dogs also show a higher cancellous and cortical bone turnover in comparison with man.² For ethical reasons, their use in research is decreasing. The pig-bone microanatomy is highly representative of human bone, as is its bone regeneration process remodeling, mineral density and concentration.²¹ However, pigs are not commonly used for bone implant studies due to notable differences regarding their bone morphology (e.g., much wider and shorter long bones in comparison with those of man), difficulties in handling, continuous weight increase, increased infection rate and difficult surgical access to long bones due to thick soft tissues layers.

Adult sheep and goats are in the weight range of humans and their long bone dimensions permit the use of human implants.² Sheep are also considered as a valuable model to evaluate human implants because of the large amount of existing information regarding the mechanical loading of their spines and major long bones, similar mineral composition to human bones as well as similar metabolic and bone remodeling rates.^{2,22}

Sheep and goats show similar bone macrostructure, microstructure, and composition though there are with very minor microstructural differences. The Haversian system is non-uniformly distributed throughout individual bones in sheep and is located primarily in the cranial, craniolateral, and medial areas of the tibial diaphysis in the goat. Primary lamellar bone is found in the caudal sector in goats.²³ These characteristics present limited variations in dogs, sheep, goats and pigs but overall remain very similar to those in man. The microstructure of dog bone has interesting similarities to

human bone, for example the presence of a secondary osteonal structure with large osteons having a diameter greater than 100 μm and containing blood vessels. However the use of dogs is generally avoided for ethical reasons. Sheep and goats have predominantly a primary bone osteonal structure with osteon diameters less than 100 μm and less visible cement lines^{4,22} and a secondary osteonal remodeling process starting at an average of 7 years.² Pigs are reported to have a lamellar bone structure similar to that of humans.²³ There are considerable macrostructural differences in the bone of rabbits and man. The microstructure of rabbit long bones also differs significantly from that of humans. In comparison with the secondary bone structure of mature bones in man, rabbits have a primary vascular structure with vascular canals of osteons running parallel to the long axis of the medullary canal.^{23,24}

Regarding bone macrostructure, few significant differences are observed between dogs, sheep, goats, and pigs. Pigs have shorter and larger bones, which are less accessible due to the thickness of surrounding muscular layers. Post-surgical infection might be more difficult to control in pigs than in dogs, sheep and goats. The small size and high cost of minipigs, as well as the continuous weight increase of domestic pigs, has limited the use of this species.

In dogs, a huge variation in trabecular bone remodeling rates can be found depending on individuals and bone sites.²³ Sheep and goats have a remodeling rate that is very similar to that of humans, supporting their use as a suitable model for testing human bone implants.^{23,25} Pigs have a bone remodeling process similar to that in man, with both trabecular and intracortical remodeling. The rabbit shows faster skeletal changes and bone turnover than man.^{23,26}

The size of the implant should be adjusted to the maximum available volume in the selected species. For example, diaphyseal implants located in the rabbit tibia or femur should not be larger than 2 mm in diameter and 6 mm in length, whereas in sheep or goats, implant dimensions can reach 4 mm in diameter and 12 mm in length. Larger implants may result in complications, such as fractures in one or multiple test sites.²⁷

Age to reach skeletal maturity should be taken into consideration before choosing a species for a bone performance study.

Life spans vary considerably between species from 2 years in the mouse, 4 years in the rat, 8 years in the rabbit, 12 years in the sheep and pig, 15 years in the dog and goat, to 30 years in baboons. Bone studies are normally conducted in adult animals. Adult animals can be defined as sexually mature or skeletally mature. Sexual maturity always occurs several months before skeletal maturity. Skeletal maturity should be preferred to sexual maturity and may be observed earlier in certain bones compared with others. It occurs between 8 and 10 months in rabbits, 12 and 16 months in dogs,

12 and 14 months in pigs, 15 and 18 months in sheep, and 24 and 36 months in goats.^{3,28,29}

The use of non-human primates and dogs raises ethical questions, and these species are most often replaced by sheep or goats; the exception is the use of baboons for the performance of very innovative intervertebral artificial disc prostheses, where this is the only recognized species.

In conclusion, the bone microstructures of dogs and pigs are as close as possible to that of man, but their use is limited by ethical (dogs) or practical (pigs) considerations. Sheep and goats show differences in microstructure but have similar bone remodeling processes making their use very common in regulatory studies. Rabbits and, to a greater extent, rodents are the least similar species to humans given the differences regarding bone repair rates, bone remodeling, and bone microstructure and macrostructure.

12.4.5 Examples of preferred models for the evaluation of bone implant performance

Based on various considerations, the preferred models for evaluating different sorts of bone implants are listed in Table 12.1. More information on these models is reported in Sections 12.9 to 12.15.

12.5 Designing a study to evaluate performance of bone implants

Thorough non-clinical evaluation is mandated by regulatory approval agencies such as the FDA, Japanese Pharmaceutical and Medical Device Agency (PMDA), Chinese State Food and Drug Administration (SFDA), European Notified Bodies, European Medicines Agency (EMA) and other worldwide authorities. These national or international agencies encourage a sequence of targeted non-clinical studies before human trials are initiated. The precise extent of these non-clinical performance testing programs is governed by experience of the authorities with equivalent devices, device class, and composition, as well as the requirements of available regulatory guidance documents. The design of a non-clinical study must be adjusted to its objective. The objectives of the study must be carefully defined and the choice of endpoints is critical in the study design. Based on the selected endpoints and study duration, the sample size necessary to achieve the desired statistical significance can be estimated.

The ideal performance study should mimic as closely as possible the clinical environment. Only results that are both quantifiable and suitable for statistical analyses can predict clinical performance and their validity be recognized by scientific, quality and regulatory authorities.

Table 12.1 Examples of preferred models to evaluate the performances of bone implants

| Bone implants | Applicable <i>in vivo</i> model |
|---|---|
| Osteoinductive materials | – Rat subcutaneous or intramuscular model |
| Bone substitutes for long bone applications | – Bone-defect repair models in the diaphysis or distal metaphysis of the femur in the sheep or goat after a preliminary screen in the rabbit |
| Bone substitutes used in cranio-maxillo facial surgery | – Skull defects in the rat, rabbit and sheep – Mandibular defects in the pig and minipig |
| Bone fracture repair devices | – Rat tibia osteotomy or ostectomy model – Rabbit ulna or radius ostectomy model – Sheep or goat ostectomy model |
| Infection prevention devices | – Rabbit subcutaneous heterotopic model (screening model) – Rabbit tibia medullary contamination and implantation |
| Debris generated by various types of implants | – Rabbit or sheep distal femoral metaphysis models are suitable to assess the particle effects on bone but not to address the intra-articular effects of particles. Other models are available for this purpose |
| Spinal cages and bone substitutes used to enhance spinal fusion | – Posterolateral spinal fusion in the lumbar spine of the rabbit (screening model) – Ventral interbody or posterolateral fusion in the lumbar or cervical spine of the sheep or goat |
| Intervertebral disc replacement devices | – Lumbar or cervical disc replacement in the sheep and goat |
| Dental implants | – Models are described separately in Chapter 13, <i>Methods and interpretation of performance studies for dental implants</i> (Dr Michel Dard) |

12.5.1 Study endpoints

The study protocol should clearly describe the main study endpoint; for example, whether the implant performs as well as or better than the state-of-the-art therapy or reference implant. At least one key performance parameter should be selected to further define the primary endpoint of the study, for example, the percentage of complete histological fusion after 2 months. Secondary endpoints can be selected to better respond to the main study question or to provide responses to secondary study questions, e.g., will use-related hazards be detected by the investigator? What is the degradation kinetic of a bone substitute? What is the bone density in the fusion area?

Performance studies designed to be submitted to a regulatory authority before a marketing application often associate safety and performance parameters combined in a single study. Such studies should comply with ISO requirements²⁷ and with relevant national standards and guidelines.

12.5.2 Study variables

Attention should be strongly focused on control of the study variables with the ethical consideration of reducing the number of animals required to achieve statistical significance and comply with the current quality and regulatory requirements.³⁰ Controllable variables include animal species and subspecies, sex, age, diet, activity, temperature, humidity, anesthetic and drug therapies, surgical procedure, implant size and variability in measurable endpoints (e.g., bone density in the defect area may vary less than mechanical test data).

12.5.3 Test periods

Typical test periods for implantation are 12, 26 and 52 weeks. Some degradable implants may require longer time periods such as 78 and 104 weeks. The use of short time periods for investigations enables a better differentiation of the performance of a test article in comparison with other products. For example, a coating designed to enhance bone osseointegration around an implant may prove superior to a comparator reference product after 4 weeks, while after 12 weeks no differences between the two products can any longer be detected. Similarly, several bone substitutes, including growth factors at different concentrations or loaded onto different carriers, should preferably be compared after a short period such as 2 weeks from implantation to maximize the differences observed between groups at shorter as opposed to longer time periods.

12.5.4 Number of articles

Regulated performance studies should include at least ten test articles and ten control articles for each implantation period.²⁷

12.5.5 Surgical site selection

Surgical site location may well be based upon the mechanical properties of the implant under investigation as well as clinical indications.

Skull cylindrical defect models are non-weight-bearing defect models, long bone cylindrical defect models are partially weight-bearing models,

whereas segmental (e.g., diaphyseal) defects are full weight-bearing models. The researcher may also define the surgical site location with regard to the indication for the product under development, for example cortical or cancellous if it is designed to heal cortical and/or cancellous bone.

12.5.6 Sensitivity of the model

Underestimation of clinical performance or incremental improvement occurs when the sensitivity of a model is low. This typically happens when the healing conditions imposed by the model are very challenging (e.g., with an extremely large critical-size defect) or when the healing conditions are too easy (e.g., test implant does not improve healing in comparison with the natural capacity of the tissues). Such issues are more likely overcome when the model perfectly matches the mode of action and targeted performance of the implant. When this is not achievable technically, non-clinical models reach their limits and the investigator should search for other sources of information, such as registries or human clinical investigations.³¹ Models may also be customized to very particular modes of action or indications. For example, a critical-size defect of moderate amplitude can be combined with local periosteum and surrounding soft tissues injuries, local devascularization, systemic treatments with corticosteroids, controlled bone necrosis, etc., with the objective of better mimicking the clinical setting and enhance the sensitivity of the model.

12.5.7 General principles for performance models

General principles to follow during the course of a non-clinical performance study are summarized in Table 12.2.

12.6 Selection of reference products and controls

In many cases, performances studies aim to demonstrate equivalence or superiority of the test product in comparison with a reference product. The reference product should be selected based on at least the following criteria: marketed for at least 2 years in the main country targeted for the introduction of the newly tested implant, free from materio-vigilance adverse events specifically linked to the product, well-known and accepted by the end users, and used for similar indications under equivalent procedures.

The nature and amount of autograft controls may vary in the different species. Autografts are not recommended in rodents due to the limited amount of bone available in this species, as well as the limited amount of cancellous bone chips. Rodents also possess a greater amount of thin

Table 12.2 Guidance for pre-clinical performance evaluation of bone implants

| Parameter | Recommendation |
|--|--|
| Species | <ul style="list-style-type: none"> – Rodents, rabbits, sheep, goat (the use of dog should be avoided) – Same species and subspecies, gender, age and weight – Minimum number of animals = 3 |
| Site of implantation | <ul style="list-style-type: none"> – Location mimicking the human clinical use conditions |
| Test implant | <ul style="list-style-type: none"> – Clinical equivalency (e.g., adjusted size but similar composition, design, manufacturing, sterilization and packaging processes) – Minimum number of test implants per time duration = 10 |
| Positive control implant/ reference implant | <ul style="list-style-type: none"> – Well established = widely marketed for at least 2 years in the same geographic area as targeted, without records of product-related materio-vigilance incidents – Minimum number of control implants per time duration = 10 |
| Duration | <ul style="list-style-type: none"> – Appropriate to study purpose and implant indication – Specified in the protocol – Allowing evaluation of local and systemic safety parameters – Covering the early stage, middle stage and final degradation stage for degradable products – Including a short term period such as 4 weeks to discriminate between implant performances – Including long term periods of 12, 26, 52, 78, 104 or more (depending on the composition and intended use of the implant) to document long term and steady state reactions and comply with international requirements – Test periods that go up to the point of absorption are needed for degradable materials |
| Techniques | <ul style="list-style-type: none"> – Fully described in the protocol – Standardized – Performed by trained personnel – Compliant with the GLP requirements |
| Radiography | <ul style="list-style-type: none"> – Preoperative radiographs to confirm normal anatomy – Immediate postoperative radiographs to verify the position of the defect, quality of fixation and implant positioning – Monthly radiographs to monitor the progress of repair – Use a semi-quantitative radiographic scoring system to qualify bone repair³² – Perform radiographs following macroscopic observation of specimens at necropsy |
| Gross macroscopy | <ul style="list-style-type: none"> – The macroscopic examination can be optimized by the use of a surgical microscope to improve magnification up to five times. Take photographs. Use this technique to search for implant debris in the surrounding soft tissues |

(Continued)

Table 12.2 Continued

| Parameter | Recommendation |
|---|--|
| Microscopic examination | <ul style="list-style-type: none"> – Local tissue effects (e.g., inflammatory reaction, degenerative and necrotic changes), osteointegration, osteoconduction, osteoinduction, bone remodeling, bone density – Use a semi-quantitative grading system to qualify the size of callus, alignment of bone segments, good positioning of the implants and fixation devices – Use a sliding digital caliper and complete the appropriate measurements – Take standardized macrophotographs of tissues – More information is available in Chapter 19 <i>Microscopic and ultrastructural pathology in medical devices</i> (A. Alves, A. Metz and J. Render) |
| Other endpoints that may be combined with the performance study | <ul style="list-style-type: none"> – Handling characteristics (e.g., injectability radioopacity at implantation, etc.) – Systemic safety toxicology data – Pharmacokinetic profile of active compound release – Residual infection – Device degradation and resorption kinetics, particle migration – Neurotoxicity, clinical and histologic parameters for implants in the vicinity of the central nervous system |
| Computed tomography (CT) | <ul style="list-style-type: none"> – Can be applied <i>in vivo</i> non-invasively and on cadaveric specimens – Provides accelerated access to results in comparison with normal turnaround times for histology techniques but with lower resolution – Resolution of CT applied to cancellous specimens can reach 8-80 μm.³² 3D image reconstructions can be obtained – Quantitative CT (based on relative attenuation of X-rays by a scanned body in comparison with attenuation by water) permits measurements of CT density, expressed in Hounsfield Units (HU) (e.g., 200–700 HU for cancellous bone) – Compare the CT density in the areas of interest of the test implant and control/reference implant |
| Mechanical testing | <ul style="list-style-type: none"> – Procedures include pushout, pullout, torque removal and indentation testing – Mechanical testing is especially important in bone implant research. Examples are described in Chapter 15 <i>Mechanical testing for soft and hard tissue implants</i> (Dr Christian Kaddick) |
| Biochemistry | <ul style="list-style-type: none"> – Use enzyme linked immunosorbent assay (Elisa), radioimmunoassay (RIA) and High-Pressure Liquid Chromatography (HPLC). Increased levels of serum osteocalcine, pro collagen peptides and bone specific alkaline phosphatase in serum are indicative of bone formation |

Table 12.2 Continued

| Parameter | Recommendation |
|-------------|---|
| | <ul style="list-style-type: none"> – Increased levels of IL-1 and TNF in tissues helps quantify an eventual inflammatory reaction and compare the inflammatory potential of implants or particles |
| Test report | <ul style="list-style-type: none"> – Animal, implant and investigators identification – Complete characterization of the test and reference implants – Environmental records – Preoperative and postoperative checks – Detailed surgical and post-surgical follow-up documentation – Justification of any deviations to protocol – Results interpretation and conclusions – Copy of the original protocol |

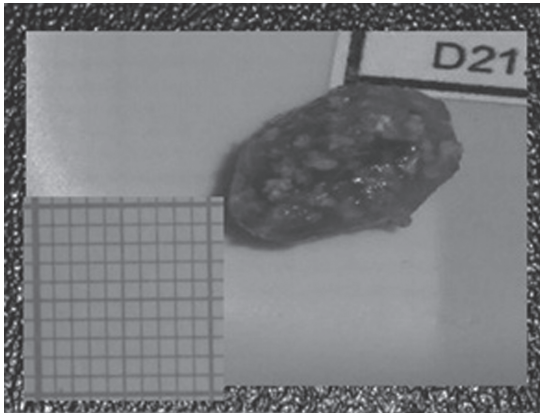
cortical bone and a thick periosteum compared with larger animal models and man. Cortico-cancellous bone autografts are more easily readily available in the dog, goat, and sheep although the iliac crest site, which is commonly used in humans, provides in large species limited amounts of autograft in comparison with the proximal head of the humerus. In these species, the proximal humerus offers an area for a large collection of cancellous hematopoietic bone marrow similar to the samples collected from the human iliac crest. In rabbits, autografts are generally collected from the iliac crest.

Other criteria for the selection of an appropriate model include:

- Anatomical similarities between the implant site in the model and in man (size and local tissue characteristics, for example, macroscopic and microscopic bone anatomy).
- Nature and amount of tissues surrounding the bone site.
- Similarity between the surgical technique employed in the study and used in the clinical application.

12.7 Osteoinductive and osteogenic performances

Osteogenic materials based on tissue-engineered scaffolds or osteoinductive materials based on bioactive growth factors and their carriers can be implanted subcutaneously, intramuscularly, intraperitoneally, and/or within the mesentery. These heterotopic sites are available in all species. For ethical and regulatory reasons, the preferred conditions are the rat subcutaneous or intramuscular heterotopic model, which are extensively described in an ASTM standard.³³ The preferred follow-up periods are 14 or 21 days depending on the nature of the tested product. Two to four sites can be tested per animal. Macroscopy is extremely helpful to evaluate



12.1 Macro photographs of a typical ectopic bone nodule 21 days following intramuscular implantation of an osteoinductive implant in the rat.

results (Fig. 12.1). Positive results (e.g., bone or cartilaginous nodules) can be confirmed by X-ray, microCT scan and decalcified or non-decalcified histology.

12.8 *In vitro* limitations

The investigator should not target only ethical approval before the start of *in vivo* studies but should first strongly focus on replacing *in vivo* by *in vitro* studies whenever it is possible. If *in vivo* studies are needed, then it is essential to reduce the number of animals to a minimum, fine-tune the protocol, and adopt appropriate surgical techniques and assessment tools in order to best profit from the data collected.

In vitro testing to evaluate bone implants involves cell culturing to provide safety information on cytotoxicity, genotoxicity, cell proliferation, and differentiation. Methods are easily standardized and are quantifiable.³⁴ For many reasons, including the lack of physiological loading under *in vitro* or *ex vivo* conditions, results from *in vitro* performance studies are difficult to extrapolate to *in vivo* situations.³⁵ When bone-scaffold and cell therapies are under investigation, the data gained *in vitro* have been shown to differ from those obtained *in vivo*.^{20,36} *In vitro* models are generally not sufficient to demonstrate performances of bone implants during advanced stages of product development. The primary goal of non-clinical animal testing is to identify implant performance problems and successes that are not assessable by *in vitro* testing, and to comply with ISO standard and National Regulations.

Three examples to illustrate the differences between *in vivo* models and *in vitro* models when evaluating bone implant performances are provided in the following paragraphs.

12.8.1 Implant corrosion testing

Many bone implants are made of metal and used as devices for procedures, such as fracture fixation, partial and total joint replacement, and bone augmentation. Corrosion, such as pitting, crevice formation, galvanic and fretting corrosion may occur. Some alloys like surgical grade type 316L stainless steel are particularly susceptible to corrosion in the environment of the body. This reduces their mechanical properties, generates particles that negatively impact implant osteointegration and releases iron, chromium, and nickel ions. These ions are known to be powerful allergens and carcinogens. Studies on retrieved human implants indicate that more than 90% of implant failures for 316L stainless steel implants are due to pitting and crevice corrosion.³⁷ When foreign materials are inserted into living systems, specific blood proteins instantaneously adsorb onto implant surfaces. This proteic layer consisting mostly of fibrinogen has the potential to inhibit corrosion and is responsible for a lower corrosion rate *in vivo* than under *in vitro* isotonic saline conditions. The range of differences observed between corrosion resistance of metallic implants and alloys *in vitro* and *in vivo* supports the idea that *in vivo* corrosion resistance experiments provide more realistic data for the evaluation of corrosion resistance of implants than do *in vitro* tests.³⁸

12.8.2 Fracture treatment in osteoporosis

The differences between *in vivo* models and *in vitro* models can also be seen in the case of products intended for fracture treatment in osteoporosis. Osteoporotic fractures are still unsatisfactorily treated with long term hospitalization, leading to the death of 20% of patients in the first year after fracture.³⁹ In an attempt to minimize the use of *in vivo* models, *in vitro* studies have been performed to evaluate the performance of devices enhancing fixation. Human cadaveric osteoporotic specimens have proved their effectiveness in mechanical testing studies to evaluate new concepts for fracture fixation. But such test systems do not take into account the bone-healing process under osteoporotic conditions, especially the decreased sensitivity of osteoblastic cells to cyclic loading and reduced remodeling cycles.⁴⁰

Cell culture based assays have been employed to assess the reaction of osteoporotic cells to different kinds of therapeutic stimuli. However, the test conditions lack not only the mechanical stimuli effects as described

previously but also the effects of the central control of bone turnover, complex cytokines, and the environment of growth hormones.^{40,41} Consequently, most authors have called for *in vivo* models to assess the performance of innovative therapies for fracture treatments in osteoporosis. The US FDA even recommends the use of two different animal models: the ovariectomized rat and a non-rodent large animal model with a Haversian system and bone remodeling process similar to that in man.⁴²

12.8.3 Challenging bone defects

Challenging bone-defect gaps are encountered in many clinical situations, such as non-union fractures, extensive therapies to treat osteosarcoma, and congenital defect. Autografting remains the most common approach. However, it is associated with high morbidity at the donor site.⁴³ Other options such as allogenic or xenogenic grafting increase rejection risks and are reported to be less osteoinductive and less prone to quick revascularization in comparison with autografts.²⁰ A lot of ongoing research on synthetic scaffolds alone (bone substitutes) or loaded with cells and/or growth factors, or bioengineered bone (e.g., bone grown under *in vitro* bioreactors or *in vivo*) requires performance models. *In vitro* methods have proved their value during the early stages of product development. However, the capacity of prototypes to enhance the development of normal bone within large defects can mainly be proved with *in vivo* models for at least the following reasons: normal bone growth requires a supply of nutrients and gaseous exchanges through angiogenesis within the repairing area and especially in the center of a large bone defect. This condition is extremely difficult to reproduce *in vitro*. Additionally, osteoprogenitor stem cells circulating within the blood stream cannot reach the defect by chemotaxis if functional blood vessels are not available. Since neovascularization is a key element to bone regeneration in large bone defects, innovative therapies cannot be thoroughly tested without the use of large animals and bone defects.^{20,30,44}

12.9 Fracture repair models

Species related differences as well as some characteristics of the most frequent methods are briefly addressed in this section.

12.9.1 General principles

By nature, the healing process shows only minor differences between species, however fractures tend to repair faster in animals than in humans. For example, in the rat, fracture healing following a standard diaphyseal osteotomy is almost exclusively accomplished by external periosteum

callus formation with very limited medullary callus involvement.¹⁷ In rabbits and larger animal species, there is a greater involvement of the medullary callus; this is also the case in man. The bone regeneration rates are in the range of 1.0–1.5 mm per day in man, 1.2–1.5 mm per day in the pig, goat, and sheep and 1.5–2.0 mm per day in the dog.²³ Mice allow for sophisticated genetic manipulation such as knock out studies, however their size makes surgical procedures almost impracticable.¹⁷ Rats are the most commonly used species in bone research, but their use is not very frequent in regulatory studies. The rat and mice models give more uniform results than large animals, because of their breeding conditions and genetic uniformity. Rats also offer a larger size compared with mice. However, standard deviations of the results tend to be larger than with rabbits and higher-order animals. These larger animals facilitate surgery and mechanical testing, and are frequently used in regulatory studies designed to confirm product performance.

Fracture fixation usually involves intramedullary nailing, plating, and screwing, external fixation or natural stabilization (e.g., radial and ulnar defects in rabbits and dogs are stabilized through the intact ulna or radius, the fibula provides some rotational stability following tibial osteotomy in rats, whereas intramedullary nailing provides flexion stability).^{17,46} When results are evaluated by radiography, semi-quantitative gradings of X-ray results are recommended. One example of a grading scale is shown in Table 12.3.

12.9.2 Methods

Fracture production in diaphyseal bones includes closed methods such as manual fractures, three-point bending methods, guillotine-like fracture systems, and open surgery osteotomies. Closed fractures reduce the periosteal stimulation and callus induced by osteotomy during open-fracture

Table 12.3 Radiographic scoring scale

| Semi-quantitative grade | Description of the callus |
|-------------------------|---|
| 0 | No callus |
| 1 | Presence of an incomplete fracture callus, with a lower density as compared to the adjacent bone |
| 2 | Presence of an incomplete fracture callus, with a density similar to the density of the adjacent bone |
| 3 | Presence of a complete callus with a lower density as compared to the adjacent bone |
| 4 | Presence of a complete fracture callus, with a density similar to the density of the adjacent bone |

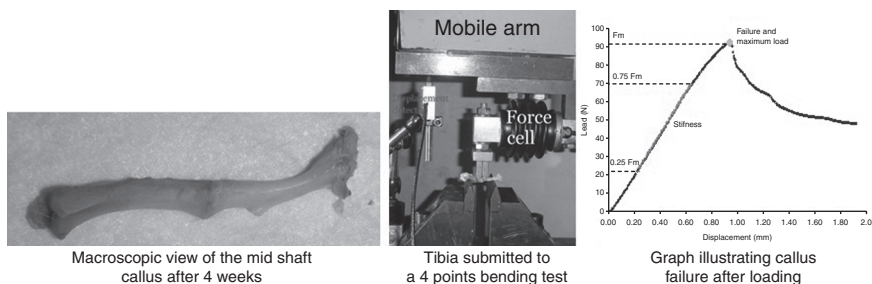
procedures. Only open-surgery osteotomies are commonly used in confirmatory studies for regulatory approval because of a lower variability in results despite the fact that they mimic accidental fractures to a lesser extent than closed fractures.

When open fractures are induced, an osteotomy or an ostectomy can be performed. The advantage of the ostectomy is to slow down and worsen the repair process to better differentiate between the performances of the tested implants.¹⁷

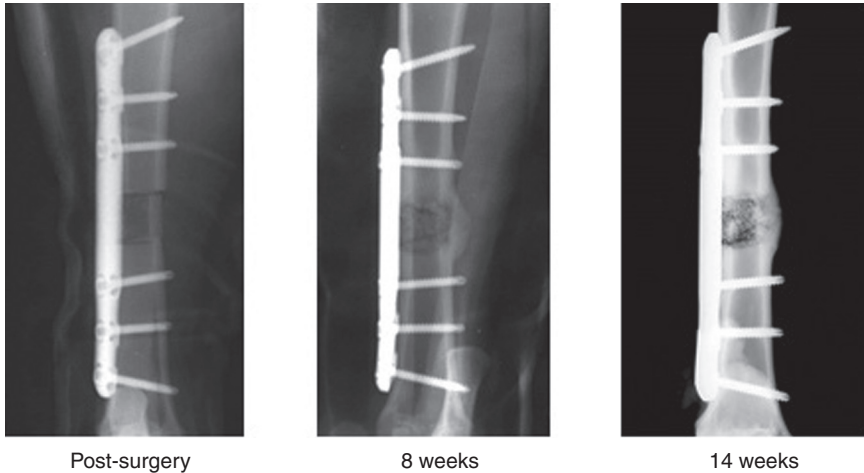
The advantages and disadvantages of the principal defect-fixation methods should be clarified.^{2,47} External fixators are often associated with pin-track infections that may negatively affect the evaluation of the soft tissue effects of new implants. Intramedullary nails are used as the standard treatment for diaphyseal fractures of lower extremities; this treatment permits central load bearing, but impairs bone blood circulation after reaming and thus is associated with prolonged periods of healing. Plate fixation is preferred even if it induces peripheral load bearing and some degree of impairment of the periosteal blood flow; this treatment contributes to standardizing fracture reduction, leaves an open space for implantation of the tested biomechanical construct, does not interfere with the defect area as much as an intramedullary nail or pin (e.g., following pin-track infection), and is the standard treatment for metaphyseal fractures.

Among the numerous fracture models available in larger species,² the rat and sheep tibial segmental ostectomy models are preferred.

The rat tibial fracture model is further illustrated in this paragraph and has proved of interest in screening implants used in bone repair therapies. It consists of a tibia midshaft osteotomy, or ostectomy stabilized with an intramedullary nail. Fracture healing is evaluated in 3–5 weeks. Complete fracture healing occurs at 5 weeks following an osteotomy and 6–8 weeks following a 0.4 mm ostectomy. Results are assessed by X-ray and mechanical testing (see Fig. 12.2) followed by histology.



12.2 Mechanical testing of a rat tibia 4 weeks after fracture treatment.



12.3 Radiographic follow-up in a plated tibial critical-size 25 mm defect in the sheep. The bone substitute tested in this model showed radio opacity that minimally interfered with the observation of the bone callus. After 8 weeks, a partially mineralized callus was observed and full mineralization was achieved after 14 weeks suggesting almost complete healing of the critical-size defect. No signs of failure were observed regarding the fixation system.

More advanced species models, such as the sheep segmental defect, are often used to confirm results and obtain regulatory approval – such a fracture model is illustrated by X-rays in Fig. 12.3. The sheep tibia segmental defect model is preferably fixed with a 3.5–4.5 mm compression plates with 8–12 holes. A plate thickness of 4.5 mm is recommended to prevent plate bending and complications.

Osteoporosis fracture

The purpose of *in vivo* models to evaluate fracture treatment in osteoporosis is to induce bone fragility, reflected by decreased bone mineral density (BMD) and mechanical properties. Large animals are principally considered to evaluate implant-based treatments because of the size of their long bones, more similar to those of humans than of smaller species. However, the use of the dog is not recommended, for ethical reasons and the strong heterogeneity regarding cancellous bone volumes following osteoporosis induction. This is so despite the advantage of dogs showing cortical bones with Haversian systems and internal cortical and cancellous bone remodeling processes similar to those in humans. In the sheep, ovariectomy alone does not produce a decrease in BMD to a similar level as that observed in man. However, long term corticosteroid treatment combined with ovariectomy

induces a delay in fracture healing in sheep tibia, with respect to callus formation, mineralization, and mechanical properties.^{40,48}

12.10 Spinal fusion models

Spinal instability is the primary cause for chronic spine-related pain and progressive neurological deficit. Spine fusion has proved effective in the management of spinal instabilities, deformities, and resulting painful conditions. Pain can be successfully controlled by the use of fusion and non-fusion implants to eliminate the painful degenerated disc.

Fusion implants refer to interbody ventral fusion cages and materials used to fill such implants, intervertebral stabilization systems, and materials used to enhance posterolateral fusion of transverse processes.

Non-fusion implants refer to total disc replacement (TDR) procedures. TDR procedures have been developed to address some shortcomings of fusion: the relative immobility of fused spinal segments transfers mechanical stress to adjacent segments, leading to an acceleration of adjacent level degeneration.⁴⁹ Additionally, the sagittal alignment of fused spinal segments cannot adapt to variations in posture.^{50,51}

The anatomies of the sheep and goats are extremely similar to that of man, and differences between the sheep and human anatomy have been carefully described.⁵² Intertransverse process fusions in rabbits and sheep have proved effective when extrapolating to posterior fusion treatments in man. Posterior intertransverse fusions are not commonly performed in rabbits and require stabilization with transpedicular fixation in sheep and goats.

12.10.1 Intertransverse posterior-lateral fusion models

The rabbit posterolateral lumbar fusion technique is commonly used^{53–55} and has often shown statistical differences between growth factors and carriers with for example 100% fusion in the growth factor group, compared with 42% fusion with the autograft group, within 4 weeks of implantation.⁵⁶ Additionally, because the fusion rate in the control group is similar to that found in human clinical studies under uninstrumented conditions, the rabbit posterolateral model is suggested as one of the most appropriate to simulate the human condition.⁵⁷

Goats and sheep are also used to induce intertransverse (posterolateral) fusion, however internal fixation is required with such species, whereas no fixation is necessary in the rabbit model.⁵⁸ In goats and sheep, following exposure of the articular processes, lateral pars and dorsal segments

of the transverse processes, decortication of the dorsal surface of each transfer process and lateral pars is performed with a high-speed burr. Cancellous bone is exposed and treatment is applied bilaterally in the area spanning the L4-L5 transverse processes and lateral pars. Illustrative images are provided in [Plate XI](#) (see colour section between pages 246 and 247).

12.10.2 Interbody ventral fusion models

Anterior or ventral fusion is not recommended in rabbits and is mostly performed in sheep or goats, with no cervical or lumbar instrumentation since stabilization does not increase fusion rates.⁵⁹

Large animal models are commonly used to study anterior fusion because technical difficulties are encountered in smaller species. There is good history of use of the larger species and it is possible to use human pediatric implants in goats and sheep. Goats⁶⁰ and sheep^{61,62} are typically used to mimic ventral fusion condition. The horizontal loading in the lumbar spine of the sheep and goat does not mimic the human conditions as well as the cervical spine, where a more vertical posture had been justifying the use of these models.⁶³⁻⁶⁵

The cervical and lumbar areas are generally used and [Plate XII](#) (see colour section between pages 246 and 247) illustrates an L5-L6 anterior interbody fusion following a retroperitoneal approach, implantation of a fenestrated titanium cage loaded with a growth factor in a collagen sponge or with a cancellous autograft in the control group.

12.10.3 Study endpoints

Results can be evaluated by monthly X-ray and CT scan as shown in [Plate XI](#). The time period should be early enough to permit the observation of differences between treatments so if for example, complete fusion occurs at 4 months in the sheep after interbody lumbar fusion, the treatment groups may show differences from controls when the histological fusion rate is considered at this time point. Manipulation of the fusion sites after sacrifice helps evaluate the mechanical fusion through the perception of motion between segments. However, this evaluation remains subjective and controversial. Mechanical testing may be preferred to measure the corresponding displacement,⁵⁷ but the most powerful tool is serial histological sectioning to determine the extent of the bone formation process, the continuity between bone trabeculae of two adjacent vertebrae, and the extent of remodeling, as illustrated in [Plate XI](#).⁶⁶

12.11 Cylindrical defect models

Cylindrical defects offer several advantages including fast surgery, standardized dimensions, absence of stabilization, reduced trauma resulting in less interference with local and systemic parameters, and easier X-ray and CT scan examination in the absence of metallic material used for stabilization as illustrated in [Plate XIII](#) (see colour section between pages 246 and 247). Depending on the test implant site of use, cranial or long bone metaphysis and epiphysis cortico-cancellous defects may be created.

In cranial defects, two cortical layers sandwiching cancellous bone are removed. Following implant placement over the dura mater, the periosteum might be retained and replaced in larger species. Species related differences are common and, as with any model, full characterization and comparison with equivalent models has proved to be helpful in selecting models that help reduce the variability of the results. The rat and rabbit skull defect models have been compared, with a lower variability observed in rats. The sheep skull defect model can be used to confirm results obtained in rats. The rat model is briefly described in [Plate XIV](#) (see colour section between pages 246 and 247).

Cylindrical defects in long bones can be made in the diaphysis (e.g., the periosteum, cortical bone, and endosteum are removed) or in the metaphysis and epiphysis (e.g., the periosteum, cortical bone and cancellous bone are removed). It has been observed that cylindrical defects in a cortico-cancellous environment are sensitive to detecting differences in performances between osteoconductive bone substitutes, osteoinductive factors and osteogenesis therapies based on osteogenic cells processing and transplantation.³ One reason may be that in such defects, tissue repair is oriented from the periphery to the center of the defect. Assuming that the study time periods are well selected, the mineralized tissue location detected by microCT (see [Plates XI](#) and [XIII](#)) or non-decalcified histology, and the mineralization line detected after fluoroscopic labeling, permit one to precisely characterize the progression of the repair process. In particular, the characterization of vascularization, bone formation, bone remodeling and implant degradation allows a more precise differentiation between candidate products.

These defects in larger species may be of sufficient size that the biological environment in the interior of the defect is characterized by profound hypoxia, which is a key feature limiting cell survival and bone repair in clinical human defects.³ For example, the sheep femur metaphyseal defect is often used to compare innovative bone substitute, growth factors, tissue engineering, and regenerative techniques. Smaller defects such as in the mouse, rat and rabbit (see [Plate XIII](#)), where distances from the edge to the center of the defect are less than 3 mm, offer much easier conditions for bone repair

due to cell survival, supported by diffusion of oxygen and nutrients from the periphery of the defect. Also, in these small defects, the diffusion gradient of growth factors is limited, impacting negatively the discriminatory power of the study when the potential of several carriers to release effective doses of growth factors is evaluated.^{3,16}

As with segmental defects, critical-size defects are selected so as to be large enough not to heal without intervention and provide a negative control environment, but small enough to make the healing parameters sensitive to a potentially minor improvement induced by the test article.³ Skull critical defects are more consistently associated with a high incidence of delayed repair in comparison to defects in the radius, ulna, or tibia because of a greater capability of the long bone periosteum to repair fracture in comparison with the calvarial periosteum.⁶⁷

The rabbit skull defect model is well characterized and recognized, and requires no implant fixation because of the support by the dura and overlying skin. The rat skull allows the creation of two critical-size defects of 6 mm diameter each as shown on [Plate XIV](#) while the critical size is 15 mm for a single defect in the rabbit model. In the rabbit, the periosteum can be closed together with the adjacent subcutaneous tissue whereas in the rat only the subcutaneous tissue can be used to close the defect. Large defects are recommended as they better mimic the clinical situation and are more appropriate for studying implant degradation kinetics. Various sizes of skull defects can be created in the sheep cranial bone with critical-size defects starting from 20 mm in diameter.

12.12 Segmental defect models

Several models of segmental defects and associated recommendations have been published by the ASTM.¹ Standardized segmental defects are often performed in the rabbit or dog radius and ulna, or in the femur and tibial diaphysis of sheep, goats, and dogs. To better reflect the clinical situation of the composition of local tissues regarding growth factors and inflammatory cytokines, defects can be created 8–16 weeks before implantation of the test product.^{20,69}

The size of the defect has been strongly emphasized as a major factor influencing the incidence of spontaneous defect healing. When the length of the segmental defect in the diaphysis reaches two times the diameter of the selected long bone, the defect does not heal spontaneously and is called a critical-size defect.

Segmental critical defects vary in size based on the species: from 0.5 cm in the rat tibia,^{69,70} 1–2 cm in the rabbit radius or ulna,^{71,72} and 3.5 cm in the sheep tibia.^{73,74} The sheep tibial defect has already been illustrated in Fig. 12.3. Of note, given the strong capacity of the long bone periosteum

to repair fractures in animals, unless it is completely removed, a large proportion of segmental defects will heal spontaneously.⁷⁵ In addition to bone resection, extensive stripping of the proximal and distal segment periosteum is recommended to achieve non-union.^{76,77}

Other factors have a critical influence on defect repair. Defects repair less well in a location that is more distant from the bone epiphysis if adjacent soft tissues are limited, in the absence of the periosteum and when rigid fixation methods are employed.^{78–80} Other techniques, such as freezing at -20°C and medullary cavity reaming, have been used to devitalize the bone ends.

Various techniques are available to stabilize experimental defects including intramedullary nailing, external fixators, internal bone plates, and screws. A careful consideration of the different techniques should be made to prevent fractures and to avoid too rigid fixation methods, since it has been shown that overly rigid fixation delays bone remodeling.⁸¹

The rabbit radial defect (see [Plate XV](#) in colour section between pages 246 and 247) should be no less than 15–20 mm long and the periosteum should be carefully dissected from the surrounding muscles. Any periosteum left in place will favor bone regeneration within 3 months. No fixation is needed and unrestricted movement is recommended. Some authors recommend ulnar defects instead of radial defects, however, the irregular shape of the ulna creates difficulties and uncertainties in the process of implant positioning and sample evaluation.^{82,83}

The preferred model is the sheep tibial segmental defect with defects of 2–3 times the diameter of the tibia²⁰ stabilized with a plate and screws. Results are evaluated by radiography, CT Scan,⁸⁴ and histological and mechanical methods such as bending stiffness and loading to failure.^{3,25}

12.13 Antimicrobial performances of implants

Implant-related infection remains a critical clinical concern. Infection not only decreases implant performance but also increases the risk of mortality more than twofold, compared to patients without infection.⁸⁵ Once established, infections are extremely difficult to treat with standard antibiotic therapies and often require surgical intervention to resolve. Implants coated with silver, antibiotics, or other agents have been developed in an attempt to reduce bacterial development following surgical contamination. Evaluating implant performance is particularly challenging because of the difficulties of consistently inducing osteomyelitis within *in vivo* non-clinical models while maintaining morbidity and mortality at a low and ethical level.

In vitro methods have proved effective for screening prototype performances. Then, standardized heterotopic soft tissue infection models can be

used to screen multiple implant performances.⁸⁶ One such model is the rabbit subcutaneous bilateral pocket implanted with a test or control implant and contaminated with 1 mL of the selected American Type Culture Collection (ATCC) strain at the selected concentration, in the range of 10⁸–10¹⁰ CFU (Colony Forming Unit/mL) and analyzed after 7 days.

Because of their susceptibility to infection rabbits are the preferred models and the rabbit tibia medullary canal infection model is more common than the distal femoral condyle cancellous bone infection model.

The rat^{87–90} and rabbit^{91,92} tibial osteomyelitis or bacterial contamination models involve a combination of tibial medullary cavity reaming, vascular sclerosis and necrosis induction by administration of 5% sodium morrhuate under a volume of 0.1–0.3 mL followed by administration of a bacterial inoculum (e.g., up to 0.1 mL) of the selected bacteria at 10⁶–10⁹ CFU. This is followed by implant insertion into the tibial medullary cavity or insertion of the implant previously incubated in a bacterial solution.

In order to reduce morbidity and mortality, local administration of bacterial inoculum can be replaced by implant immersion in a bacterial suspension to create a biofilm before insertion into the tibia.⁹³ Preliminary implant contamination increases infection rates while the bacterial concentration of the inoculum can be decreased to 10²–10⁴ CFU per mL to reduce morbidity and mortality rates.

After a short period of time (e.g., 2 weeks or up to 28 days), osteomyelitis or residual bacterial contamination can be estimated through gross macroscopic assessment (e.g., swelling, erythema, sinus formation, and observation of sequestra), radiographic scoring of bone resorption, demineralization, periosteal reactions, new bone apposition, sequestra formation, tibial diameter widening, microbiology, and histology.^{92,94}

Goats and sheep have been used to induce localized osteomyelitis with pathological changes limited to the proximal metaphysis of the tibia.^{95,96} Following placement of a small drill hole in the proximal tibia, 5% sodium morrhuate is injected in a volume of 0.1 mL followed by the same volume of *S. aureus* suspension with a concentration of 10⁸–10⁹ CFU per mL. The drill hole is closed with bone wax. Osteomyelitis can be induced in 96% of the cases as demonstrated by radiographic or histologic scoring, and confirmed in 82% of the cases by positive bacterial cultures.

12.14 Bioabsorbable and biodegradable materials

A variety of materials with a bioabsorbable potential are used in orthopedic surgery (e.g., polyhydroxy acids: polylactid, polyglycolid, polyglycolid-colactid, PGA-PLA, poly(glycolid-co-trimethylene carbonate), poly(p-dioxanon), PDS, polyhydroxybutyrate, polyhydroxyvalerate, natural biopolymers (collagen, hyaluronans, chitosans, etc.), degradable medical

grade polyurethanes, calcium phosphate based materials (calcium sulfate, tricalcium phosphate, various forms of hydroxyapatite, etc.) and degradable alloys (magnesium based alloys, etc.). Standard subcutaneous or intramuscular dorsal implantation methods are recommended to provide a first *in vivo* estimation of the degradation pattern and inflammatory potential of implants. No differences are normally observed between subcutaneous and/or intramuscular implantation methods with regard to the kinetics of degradation or local tissue effects around implants. Results are evaluated by gross macroscopy, surgical microscope dissection, and implant site histology following paraffin or resin embedding procedures. When degradable implants are susceptible to generating small particles, the histological examination of local lymph nodes, spleen, and liver is recommended.

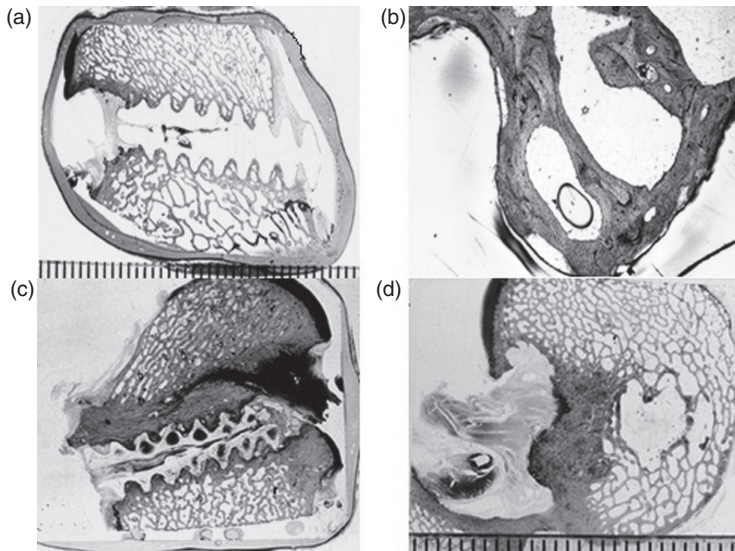
Soft tissue tests offer a quick screen of the inflammatory reaction to the implant and have proved effective for eliminating implants with local tissue incompatibility very early in the development process.

Successful products following soft tissue implantation should be submitted to a biodegradation study following bone implantation.

Following soft or hard tissue implantation, additional implants should be available in order to be retrieved after sequential periods and submitted to mass degradation measurement, chemical characterization techniques, and strength testing through bending, tensile and indentation tests.³² The principles and models described elsewhere in this chapter are often used to evaluate bioabsorbable materials, but supplementary time periods are necessary to assess the biodegradation rate of such materials as illustrated by [Plate XVI](#) (see colour section between pages 246 and 247) and Fig. 12.4.

12.15 Bone debris interaction with implant performance

Bone debris may be generated by implants and may exert deleterious local effects as well as effects in distant organs such as the liver and spleen.⁹⁷ When the presence of debris is suspected, local lymph nodes, liver, and spleen should be submitted to histology to allow the detection of particles, distant inflammatory or degenerative reactions. This is especially important for nanoparticulate products. Locally, debris may induce an inflammatory response negatively impacting the implant performance. The long-term inflammatory potential of bone implant debris can be evaluated through debris preparations mimicking the clinical conditions of implant use, debris implantation in a standard non-critical-size cortico-cancellous bone defect, cytokine dosages and non-decalcified histology to quantify the local inflammatory reaction.



12.4 PLA based bioresorbable interference screw designed for anterior cruciate ligament repair procedures – bioresorbable implant – non-decalcified histology. (a) Interference screw in the femoral condyle of the sheep after 26 weeks. (b) Details of bone trabecules in close contact with the plastic screw after 26 weeks. After 1 year (c), signs of degradation of the PLA are visible while the bone to implant contact remains of a high grade. After 3 years of implantation (d), the plastic screw (totally resorbed) was replaced by dense cortical bone and bone lacuna while signs of attachment of the transposed ligament to bone can be clearly identified.

12.16 Conclusion

Non-clinical performance models may be criticized because they sometimes tend to overestimate performance in comparison with the human clinical experience and because they are not all equal in their capacity to predict clinical efficacy in man. A large number of sophisticated *in vivo* models are available. However, model characterization studies show that only a small number of these models provide reliable results and an even smaller number of models are officially recommended in standard and guidance documents. Other models require some degree of customization before use.

Several working groups on standardization are involved in the selection of reproducible, sensitive, and specific models. Difficulties are encountered, not only due to the variability and complexity of living test systems, but also due to the multiple sources of variations (e.g., surgical technique, animal species and subspecies, genetics, age, diet, activity, sensitivity and specificity

of the selected observation methods) introducing difficulties in the characterization and comparison of the models. The most direct consequence for the medical-device manufacturer is a risk of misinterpretation of the study results and underestimation of the performance of the newly developed bone implant. Such risks might be controlled, not only by improving standardization, but also by rigorous documentation of standard operating procedures, training (records), historical data obtained with the same model, interlaboratory trials, and exchange of information.

Those models that tend to overestimate performances in comparison with the human situation may still have value in helping to identify minor improvements. As an example, when the performance of recombinant bone morphogenetic proteins (rhBMP) is under investigation, the rabbit ulna and radius segmental defect models are suspected of overestimating clinical performance⁹⁸ because of rapid healing of rabbit bones in general due, possibly, to a greater number of osteoprogenitor cells and their more intense reaction to rhBMP-2 in this species.⁹⁸ In this model, rhBMP-2 and its carriers have often shown strong acceleration of healing (up to 33%) and improvement of mechanical properties (up to 100%) 4 weeks after fracture treatment in comparison with sites treated with a carrier impregnated only with a buffer and sites left untreated.^{99,100} RhBMP-2 in a buffer vehicle has shortened time to healing by up to 50% in comparison with untreated sites in a rat osteotomy model.¹⁰¹ Such accelerations of the healing time obviously overestimate clinical performance and may be observed in rodent and rabbit fracture models or in larger/more advanced species when the pathological conditions (experimental injuries) are set at a minimal level. A typical example of minimal injury induced in a highly advanced species would be the fibular osteotomy model in non-human primates: an rhBMP-2- α BSM formulation resulted in a 40% acceleration of fracture healing at 10 weeks compared with the healing of untreated osteotomy sites.¹⁰²

Because of their great sensitivity, models such as these (e.g., small species under any conditions or large/advanced species under minimal pathological/defect conditions) are only indicated to compare formulations or prototype candidates for further development. Once the best candidates are selected, non-clinical performances should be confirmed in a model that better predicts the human clinical performance and involves more challenging conditions. Easier conditions create sensitive models, with a capacity to discriminate different product candidates for further development and to help eliminate poorly performing candidates at an early stage.

Nevertheless, no model is ideal and models have their own characteristics, thus selection must be based on the scientific questions raised in the study. In these conditions, some non-clinical studies accurately corroborate the results of human trials. For example, most large animal non-clinical studies have accurately predicted the outcome of phase 2 and phase 3 randomized

controlled human trials for many products including biologics such as FGF-2 and PDGF-BB.¹⁰³

Customization of non-clinical models (e.g., modulating the host defense, mimicking systemic health impairment status such as diabetes mellitus, smoking, osteoporosis, etc.) also allows for more targeted and refined human performance prediction.

The use of well characterized, standardized or customized models conducted in compliance with the principles of Good Laboratory Practice provides data of high quality, allowing comparisons to be made with historical data, a reduction in the number of animals required, and increased capability of identifying performance limitations and opportunities. In a central place between early bench testing and the final bedside challenges, *in vivo* performance models contribute to advancing patient treatment outcomes.

12.17 References

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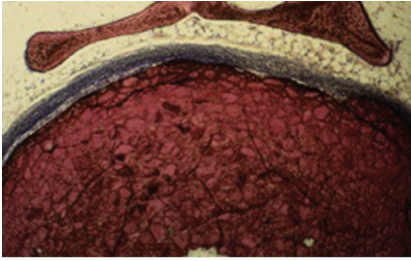
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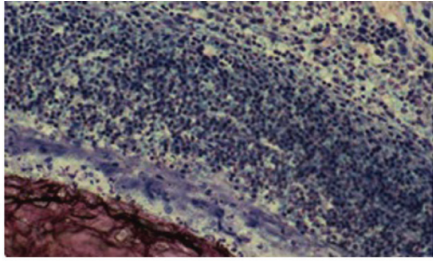
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(a)



(b)



(c)

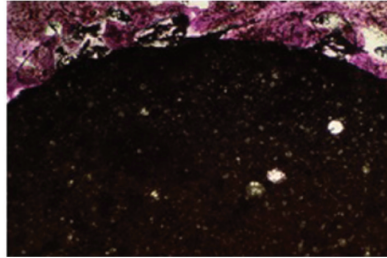


Plate IX (Chapter 12) This chitosan based bone substitute showed poor osteointegration performance. An osteolytic process was induced. It resulted in the absence of osteointegration and reduced bone density in the vicinity of the implant as shown on this non decalcified histology picture from specimens collected 6 months after implantation. Additionally, the implant degradation performance was graded poor with no visible signs of significant degradation of the product (a). The chitosan product contained impurities that were released into the surroundings of the implant and activated a strong lymphocytic and macrophagic inflammatory reaction (b). In comparison, a reference implant showed good osteointegration performances and no inflammatory response after 6 months of implantation (c).

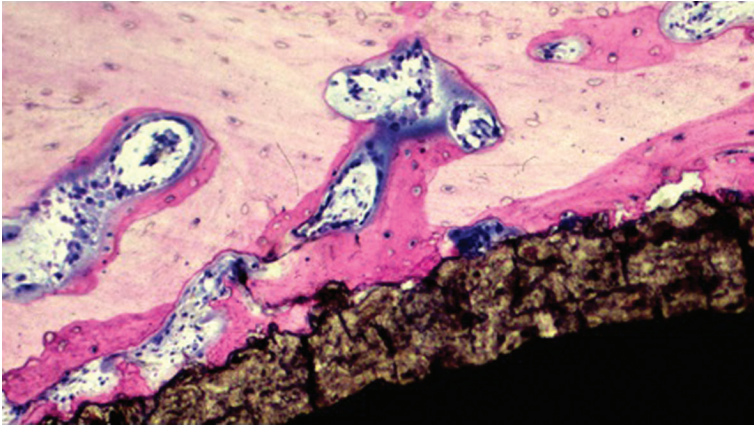
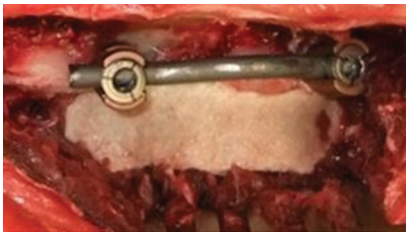
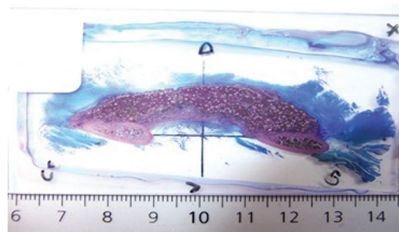


Plate X (Chapter 12) High magnification microphotograph illustrating osteoconduction and osteointegration of a bone implant coated with hydroxyapatite. The image shows an interface between a metallic implant and its hydroxyapatite coating, as well as an interface between the hydroxyapatite coating and the host bone. In this image, the newly formed host bone covers most of the surface of the coating and lines of active osteoblasts which actively synthesized the osteoid substance.

(a)



(b)



(c)

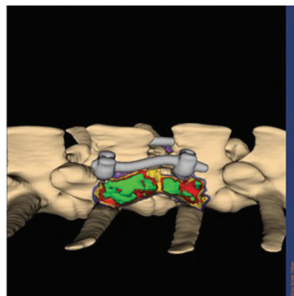
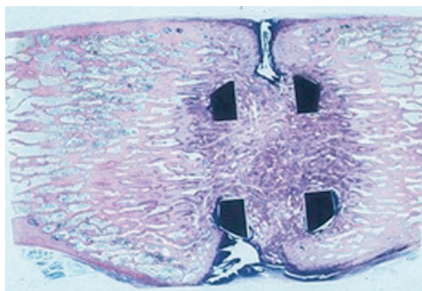


Plate XI (Chapter 12) Illustrative images of the sheep postero-lateral fusion model. Operative view immediately following transpedicular fixation and test implant (white) positioning (a). Under non-decalcified histology, a mixed osseous and mineral scaffold (whitish granules) bridged two lateral transverse processes 12 weeks after surgery (b). 3D reconstructed quantitative CT was used to measure bone densities (expressed by color codes) and to compare test and reference control implants performances. Panel (c) shows incomplete fusion and partial osseous bridging between two adjacent vertebrae 10 weeks after fusion induction.

(a)



(b)

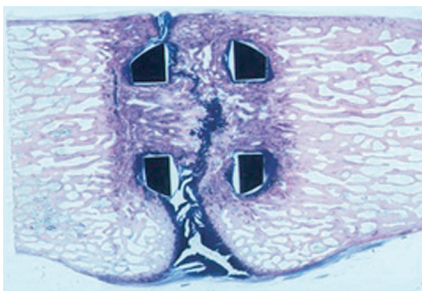


Plate XII (Chapter 12) Non-instrumented ventral interbody fusion in the sheep – non-decalcified histology. Three months following fusion induction with an interbody cage loaded with a growth factor mixed with collagen (a), complete histological fusion was obtained. The two adjacent vertebrae showed continuity between cancellous bone trabeculae. In the reference control group (b), the cage was filled with a cancellous bone autograft. The image shows non-fusion with the presence of a bluish cartilaginous line separating the two adjacent vertebral bodies.

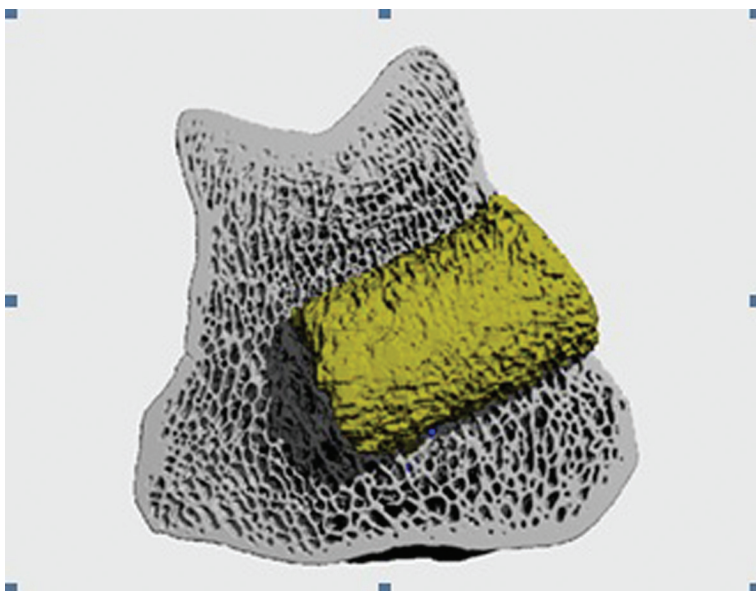


Plate XIII (Chapter 12) 3D micro-CT scan reconstruction showing a 5 mm wide cylindrical cancellous defect in a rabbit, filled with a bone substitute. Immediate postoperative evaluation following defect repair with a phosphocalcic bone substitute.

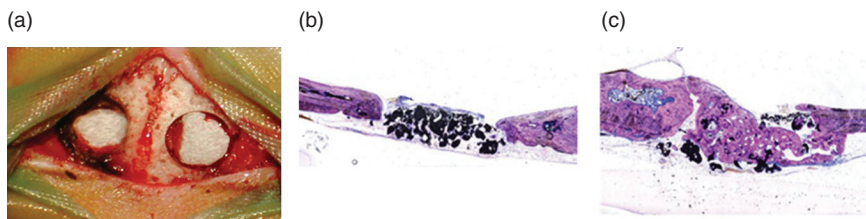


Plate XIV (Chapter 12) Macrophotograph illustrating the results from a rat calvaria defect model. Twenty-eight days after surgery and implantation (a), the skull was sampled for gross macroscopy and radiographs. Non-decalcified histology revealed that the mineral bone substitute alone used as a negative control was not osteointegrated after 28 days (b), whereas the test implant impregnated with a growth factor (c) was fully osteointegrated and the implantation site was filled with newly formed cancellous bone.

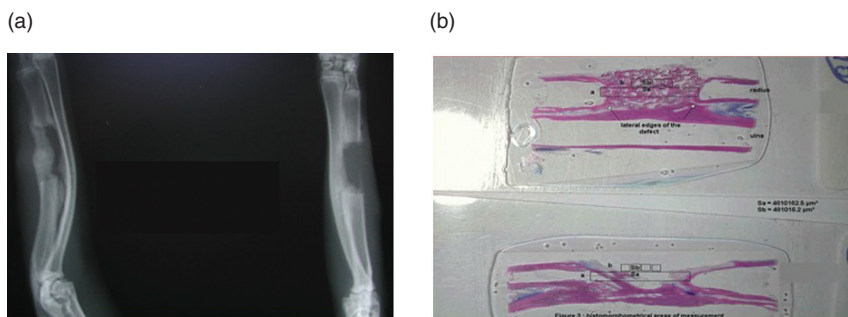
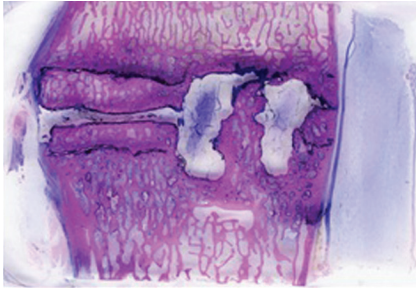


Plate XV (Chapter 12) Rabbit radial critical-size defect after 4 weeks. (a) The radiographs indicate the presence of an almost complete fracture callus, with a density similar to that of the adjacent bone on the left side which was treated with a scaffold loaded with a growth factor (test implant), whereas no callus was observed in the right side treated with a scaffold alone (negative control). (b) The upper non-decalcified histological slide shows a high bone density in the defect area in the growth factor treated group whereas the lower slide shows that no bone developed in the central area of the defect. The frames indicate the area of interest for histomorphometrical measurements.

(a)



(b)

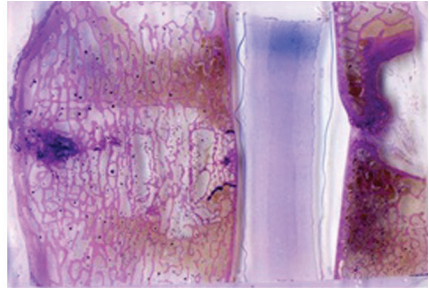


Plate XVI (Chapter 12) Non-instrumented ventral interbody fusion in the sheep – bioresorbable implant – non-decalcified histology. A bioresorbable PLA cage showed early signs of degradation long after completion of histological fusion (a). Thirty-six months after implantation, no signs of residues of the cage were visible. The presence of bone trabeculae outlining the original contour of the cage allows the recognition of the original implant site. Thirty-six months after implantation and approximately 33 months after fusion, the implant is completely resorbed (b).

Methods and interpretation of performance studies for dental implants

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Abstract: This chapter proposes a methodological approach to investigate the biological performance of dental implants. The rationale for the decision on animal and experimental models is discussed. The general structure of an animal trial protocol coupled with essential guidelines on organizational issues is given. The related portfolio of convergent analytic methods and the manner to implement them is proposed.

Key words: dental implant, biological testing, animal models, bone, soft tissues.

13.1 Introduction and definitions

A dental implant is a medical device implanted into the patient's body as surgical treatment for a tooth loss. This artificial tooth-root is used to support abutments and dental restorations, such as crowns, bridges or over-dentures.

The great majority of dental implants placed today are root-form endosseous implants. Prior to the advent of root-form implants, most were either blade implants (in that the shape of the metal piece placed within the bone resembled a flat blade) or subperiosteal implants (in which a framework was constructed to lie upon, and was attached with screws to, the exposed bone of the jaws).

During the pioneering years of implant dentistry attention was paid mainly to the shape of the implant and, as a consensus on the root-form emerged, the focus of interest progressively moved from the macro-cosmos (general shape), through the micro-cosmos (surface structuring), to the nano-cosmos (surface functionalization). Nowadays a synergistic approach of these three levels of tissue integration remains necessary and this renders scientific investigation of dental implants more complex. The evaluation procedure starts by examining the device with isolated cells *in vitro*, and continues by implanting it into multi-cellular organisms *in vivo*.

It is likely that the trend of the future will be to integrate cells *in vitro* investigations and animal *in vivo* trials, which will complement each other within an interactive research program.

Strategically positioned between materials science and clinical research, preclinical research offers a necessary field of expertise to investigate dental implant behavior in contact with living organisms (cells or animals) in order to assess and understand the host response.

Successful performance is the accomplishment of a given task to preset standards of accuracy, completeness, fastness and cost, and the objective is that a dental implant fully replaces a functional tooth.

Within this context a proper evaluation of the performance of a dental implant implies that investigations are conducted with devices in their final shapes, dimensions and surface structuring. These evaluations end up with long-term prospective and controlled trials in humans. Preclinical studies, particularly in animals, constitute a prerequisite to accumulating knowledge under reproducible and standardized biological conditions, progressing to human trials at a later stage.

All animal trials must comply with the highest standards in experimental surgical research and comparative medicine. By the definition established by the Academy of Surgical Research (Eden Prairie, MN, USA), experimental surgical research is that part of surgical research that examines and evaluates, in animals, both innovative and conventional ideas related to surgery through scholarship analysis of data and the generation and investigation of hypotheses. This is key to establishing the originality of the scientific question leading to this working hypothesis.

13.2 Importance of performance evaluation studies for dental implants

The three levels of tissue integration (macro-, micro-, nano-), and their consequences on living organisms, can only partially be observed and understood in animals, although the contribution of cellular and molecular biology remains essential for two of these three levels, namely the micro- and nano-cosmos.

Consequently a performance study assesses the behavior of a dental implant and its effects on the host tissues after implantation in an animal anatomical region whose properties closely resemble the human implantation area.

Dental implant surgery in animals is a survival surgery, which penetrates and exposes a body cavity, or produces substantial impairment of physical or physiological functioning, or involves extensive tissue dissection, or has the potential for producing a permanent handicap in an animal. Therefore, the highest awareness must be raised by all involved parties.

The ISO 10993-6 (2007) covers completely the topic of biocompatibility testing for implant devices, and in its chapter 5 barely address the evaluation of the performance in animals.

According to the ISO 22911 (2005), where the test animals are defined in paragraph 4.2.2, no particular animal model of a usage test for dental implant systems has yet been validated as relevant to the human situation. It is recommended, therefore, that an animal species be chosen that meets the following criteria:

- (a) oral hygiene can be maintained, either naturally or artificially;
- (b) the jaws are of sufficient size to allow normal surgical access and to accommodate the dental implant system in its form intended for use in humans;
- (c) the site into which the dental implant system is to be placed should have opposing teeth;
- (d) the animals should be skeletally mature if appropriate for its intended use;
- (e) animals having a non-herbivorous pattern of masticatory jaw movement are preferable.

The above recommendations restrict the types of model to adult swine, canines, and non-human primates and, in particular cases where the teeth functional loading or the pattern of mastication is not important, to adult sheep and goats.

We underscore the imperative refinement that the ISO 22911 is extended to consider the surgical access, which can be extra- or intraoral into the model.

The resulting matrix shows that swine, dogs, and non-human primates can benefit, like humans, from an intraoral surgical approach for inserting ‘human-sized’ dental implants into an intraoral environment, allowing assessment of their performance in jaw bone and/or soft tissues (Table 13.1).

Furthermore, these three animal models allow for a two-phased sequential approach – phase 1: investigation into the basic properties required for a dental implant (i.e. osseointegration) – phase 2: investigations into a model in which particular clinical indications and surgical procedures are associated (i.e. osseointegration in irradiated mandible sites). In phase 2 the

Table 13.1 Decision matrix on animal species

| | Extraoral biological environment | Intraoral biological environment |
|-----------------------------|--|----------------------------------|
| Extraoral surgical approach | Mouse, rat, rabbit, goat, sheep, swine, dog, non-human primate | Rat, rabbit, goat, sheep |
| Intraoral surgical approach | – | Swine, dog, non-human primate |

studies are improperly described as ‘clinically relevant’ when clinical situations and materials normally used in human clinics are being investigated. We prefer to use the terms of pre-translational and translational experimental surgical research for phases 1 and phase 2, respectively.

It is important to keep in mind that the results, as they stand, obtained from an animal trial, although translational, do not allow any direct correlations and conclusions concerning human situations or treatments to be drawn.

Nevertheless, well-designed animal studies, and consequently properly implemented animal models, will enhance translational prospects for the corresponding human surgical treatment of interest.

An animal study finds its strength if correlated in advance with other animal trials belonging to the same synergic program. This implies a progressive and incremental approach including studies with different animal and experimental models that complement each other and allow discrimination through comparison.

13.3 Experimental design of a performance trial for dental implants

13.3.1 Principal investigator and quality management system

The principal investigator (PI) assumes the full leadership for a defined study from a research institution, a university, a contract research organization, or a corporate sponsor. He/she supervises the elaboration of the protocol, applies to the Institutional Animal Care and Use Committee (IACUC), vouches for the calibration of the operators, ensures the organization and logistics of the surgical sessions, and coordinates the interpretation and the presentation/publication of the results.

The PI complies with the regulations and obligations dictated by the quality management system in place at the surgical research facility performing the animal trial. Consequently the PI applies and controls full adherence to the 3Rs (Replace, Reduce, Refine) principle (Russell and Burch, 1959) as to all national and international laws, regulations and policies pertaining to the welfare of laboratory animals.

The PI relies on the highest proficiency in accordance with current best practice in animal trials management, experimental surgical research and, most relevantly, in-life and post-mortem testing.

It must be stressed that researchers (surgeons, staff) conducting surgical procedures should complete an appropriate training to ensure that good surgical technique is practiced.

It is recommended to include also an educational apprenticeship process, whereby a non-experienced surgeon assists a fully experienced one.

13.3.2 Animal trial protocol

This section presents the general structure of an animal trial protocol, coupled with the main recommendations in relation to the successive sub-sections. It should help readers to prepare their own protocols according to the best practice, and assist in the care of animals in ways that are scientifically, technically, and humanely appropriate.

13.3.3 State of the art and rationale

The aim here is to examine and critically discuss existing review papers related to the scope of the study, key research publications having a high citation index, and scientific contributions that have paved the way for the proposed study. This part emphasizes the originality and importance of the scientific question ultimately leading to the working hypothesis.

Hypothesis

The research hypothesis is both the basement- and the key-stone of a study, and conceptually it exists as several types (Toledo *et al.*, 2011).

In practice, following formulation of the scientific question, a hypothesis will be formulated only after a critical analysis of peer-reviewed scientific literature. This will ensure that the focus of the study is original and does not duplicate other studies.

The hypothesis should be self-explanatory, clearly written, and understandable at a first lecture. It should not contain more than one statement, and relate to the statistical analysis planned for the study, in particular terms, such as 'similar', 'equivalent', 'superior to', 'non-inferior to' and 'inferior to', have to be sustained by the statistical power calculation (see Section 13.5).

Objectives

The objectives refer to the practical target to be reached and articulate the material element that will be investigated through the analytical procedure (i.e. histology/histomorphometry) to be used. These objectives can be set out as first, second, and possibly third if necessary.

Variables

Primary variable

A clear and easily understandable definition of the primary variable is mandatory as it constitutes the basis for the statistical power calculation and consequently for the number of animals included in the study. This variable is unique and always quantitative.

Secondary variable(s)

Generally a secondary variable is defined as a complement to the primary variable. If judged necessary, two secondary variables can be defined, and ranked in order of priority. These variables are also quantitative.

Complementary variables

Several complementary variables can be listed. They are preferably quantitative, but may be semi-quantitative if founded on widely accepted principles (e.g. histological grading from the ISO 10993-06).

13.3.4 Animal/experimental model and animal management

Choice of an animal and/or experimental model

The discussion about the respective places of the concepts of animal model and experimental model is not a theoretical one but holds very clear consequences for the design of the trial and implications on the practical conduct of the study.

The *animal model* refers to a non-human living animal with an inherited, naturally acquired, or induced pathological process or lesion allowing the resolution of a research hypothesis, and resembling a similar condition in the target human species (adapted from Hau *et al.*, 1989).

Beyond the animal model itself, but part of it, the *experimental model* is defined as the association of an animal type used for a particular surgical procedure that is to elicit a particular defect at a specific anatomical site.

The choice of models has to be argued by describing the anatomical and physiological adequacy of the animal for the foreseen surgical procedure.

Within the framework of the present chapter, the balance between porcine, canine and non-human primate will be discussed.

In this paragraph the description of the model does not need to be extensive, but rather concise, supported by strong evidence taken from the existing literature. It has to be demonstrated that the best existing animal model will be recruited, and the most adequate experimental model will be used.

Moreover it has to be clearly stated that to the best knowledge of the PI, the intended study should not duplicate an already existing one.

Animal management

It is important that the animal study population is homogeneous in race and species, and also in gender, age and weight.

The facility has to be clearly pinpointed and the following welfare conditions described: conformity with the local, national and international regulations, the time of acclimatization and housing, the anaesthetic and pain-relief procedures, and the frequency of veterinary medicine consultations concerning animal welfare and related procedures.

This part identifies the governance of the IACUC and ensures that an uncontrollable risk of impaired animal treatment cannot occur.

The criteria for study abortion (i.e. extensive infection in more than 15% of the animals) and animal exclusion (general sickness not related to dental implant placement) should be listed. It should be referred to in the written procedures in place giving guidance on premature termination of an animal or study abortion.

13.3.5 Materials and methods

Materials

Test(s)

It is necessary to disclose extensive information on the dental implant under investigation, such as: the macro-, micro-, nano-scopical aspects, the chemistry, the type of coating, the packaging and sterilisation methods, the labeling (lot and reference number) and if applicable concentrations of active substances.

Positive control

The positive control will be a dental implant meeting the state of the art with a sufficient record of publications in preclinical and clinical trials. Complementarily to other placement conditions, its insertion in pristine bone is recommended.

Negative control

Ideally this is the opposite of the positive control, theoretically a dental implant with features that are considered inadequate with respect to the design of the planned experiment. In practice it is difficult to ascertain the required parameters for a dental implant serving as a negative control.

Methods

Groups under investigation

These are not automatically linked to the tested materials. The groups differentiate themselves from the materials as they can relate to a special surgical approach or a particular anatomical configuration.

Study design and schedule

Two types of study exist in experimental surgical research: pilot or pivotal.

The *pilot study* addresses an explorative phase, which is anticipated to optimize a new surgical approach or support the establishment and definition of the primary variable. The pilot study constitutes the critical part of a model validation, and is seen to power the definitive study design. Statistical significance is not required, therefore the number of animals is fixed between three and six. Nevertheless, the structure of the protocol is the same as for a pivotal study.

Before entering into, or in parallel with, a pilot study, cadaver tests which help to simulate and refine the surgical approach could be useful.

The *pivotal study* is designed to confirm or overturn, clearly and beyond doubt, its own working hypothesis.

At this stage of the present section the Animal Record Form (ARF) directly related to the intraoral surgery shall be established. The intraoral approach implies that two successive surgical sessions become mandatory: the first one for the teeth extraction, the second one for the dental implantation itself.

In the two following sections, which deal with the 'Pre-surgical' and 'Surgical phase', the items to be listed in the ARF will be marked by the sign [ARF]. The items relating to the 'Post-surgical phase' are generally documented in the veterinary medical file of each of the recruited animals, but exceptionally it could be easier to copy some of these data into the ARF.

Pre-surgical phase

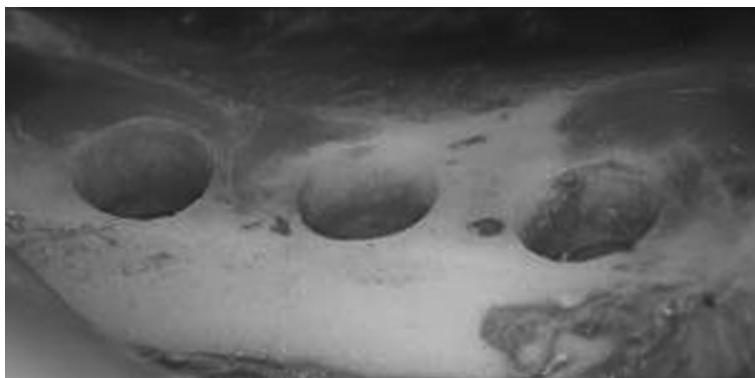
The time and duration of fasting before surgery should be defined [ARF].

A detailed description of the anaesthetics regime, including type, dose, duration and route of administration of pre-anaesthetics, anaesthetics and analgesic agents and tranquilizers/sedatives, should be presented [ARF]. An adequate antinociception must be ascertained as part of the survival surgery and occurs at the surgical plan of anaesthesia prior to surgery. This pre-emptive analgesia (preoperative and intraoperative) leads to more stable animal and optimal postoperative care.

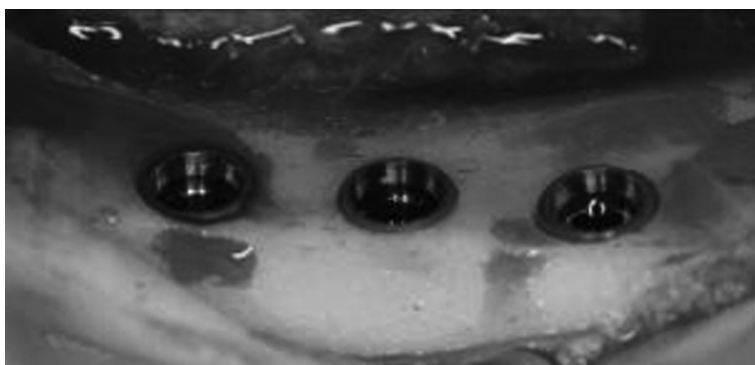
The type, amount of food and beverage, the light-time sequences in the animal stables, and the levels of temperature and humidity, should be fixed.

Surgical phase

The surgical procedure should be explained step by step, including a full description of each step illustrated by drawings (Figs 13.1–13.3).



13.1 Implantation-step 1: preparation of the dental implants beds.



13.2 Implantation-step 2: dental implants after insertion.



13.3 Implantation-step 3: abutments in place on the dental implants.

The schedule includes information on:

- the mode of aseptic preparation and maintenance for the operation suite, the animal (i.e. shaving) and the surgical area (i.e. local skin disinfection around the mouth with tincture of iodine) [ARF],
- the intraoperative monitoring, which should include routine recording of anaesthetic depth, maintenance of body temperature, fluid supplementation, cardiac and respiratory rate [ARF],
- an estimation of the duration of the surgical phase starting at first cut and ending up at sutures closure [ARF],
- the type, dose, and frequency of injection of the local anaesthesia [ARF],
- the references of the surgical instruments and equipment used (manufacturer, brand name, lot number, etc.) [ARF],
- the shapes and dimensions of the defects and dental implants, and the procedures of cooling and rinsing (i.e. with saline) during the drilling preparation of the implant bed [ARF].

At this point it becomes mandatory to consider the concept of critical-size defect. According to Schmitz and Hollinger (1986), defects that do not heal during the lifetime of the animal may be termed critical-size defects. This well-established definition forces investigators to recognize a two-dimensional perspective, in which both dimension and duration are fully considered when it comes to defect size and its follow-up:

- the type of suturing material, and the suturing method (i.e. interrupted stitches) [ARF],
- the pain relief method used when leaving the operation suite (i.e. application of trans-dermal patches) [ARF].

The role of computer-guided template-based implant placement, which shows a high implant survival rate ranging from 91% to 100% in humans, deserves to be evaluated in experimental surgical research procedures too. Preclinical and clinical studies indicate a reasonable mean accuracy with relatively high maximum deviations in image-guided-surgery. There is not yet evidence to suggest that computed-assisted surgery is superior to conventional procedures in terms of safety, outcomes, morbidity, or efficiency (Jung *et al.*, 2009; Schneider *et al.*, 2009) but it is the role of experimental surgical research to contribute to this decisive evolution.

Post-surgical phase

Some critical aspects of the post-surgical phase are common to all the experimental surgical research procedures, such as:

- The conditions of transport (inside the same building or from the aseptic surgical suite to the housing building).
- The type, dose and duration of fluid supplementation and of antibiotic treatment.
- The pain relief prescription during the days following the surgery.
- The schedule of behavioral observations by the animal-care specialists. Because animals cannot express pain or discomfort effectively, in many instances this aspect is critical, as are the related alert and/or emergency procedures to communicate concerns and/or abnormalities on health, behavior, and wellbeing to the veterinarian. The veterinarian should have access to all the medical and experimental records and moreover have authority to treat, remove from experiment, relieve pain/distress or euthanize animals.
- The procedure for termination of an animal in case of incurable health troubles directly or indirectly related to the surgery or not, should be set up and the rules for decision, implementation, and recording clearly stated.

Because of uncontrolled animal chewing, some other aspects are relevant to implant dentistry and attention must be paid to:

- The bedding (wood chips, straw, flooring) and cage equipment (bars, chains, feeders).
- The type and amount of food. A soft food diet is mandatory after dental implant insertion into the mouth of the three species of animals presented in this chapter.
- The oral hygiene measures including intraoral observation periods, prescription and follow-up of special techniques for hygiene maintenance (i.e. teeth brushing method, application of a chlorhexidine solution) [ARF].

Observations and analysis

In this paragraph the analytical methods which are foreseen to be implemented (see Section 13.6) shall be briefly described.

A non-exhaustive list contains information on the following aspects:

- Clinical observations: photographs, periodontal probing, resonance frequency analysis.
- Imaging technologies: X-rays, parallel confocal scanning, computerized tomography (CT), microCT, etc.
- Histology histomorphometry complemented if necessary by immunohistochemistry techniques, contact micro-X-rays, electron microscopy observations, etc.

- biomechanical investigations, such as removal torque, push out or pull out testing.
- Tissues gene expression.

13.3.6 End-points and terminal procedure

The termination shall be conducted humanely according to a written procedure in an operation suite dedicated to this act. It is mandatory that the method of euthanasia, including agent, dose and route, ascertains and adheres to the most current guidelines on animal euthanasia.

The sample harvesting procedure will be described step by step indicating the types of instrument, the chemicals as the duration of their use [ARF].

13.3.7 Expected results and statistical power calculation

This paragraph has to be agreed with, and finally written by, an entrusted professional statistician who shall be involved at a very early stage of protocol preparation (see Section 13.5).

This statistician is in charge of the preparation of the randomization schedule, which is a part of the ARF [ARF].

13.3.8 Timelines

A time table shall summarize all the procedures, from acclimatization to termination, outlining the milestones.

13.3.9 Reporting

The dates for reporting (interim and final) and submission of manuscripts to peer-reviewed journals should be estimated. It is recommended to apply the ARRIVE (Animal Research Reporting of *In Vivo* Experiments) guidelines in preparing the manuscripts (Kilkenny *et al.*, 2010).

13.3.10 Data recording and archiving

For each animal, the following data should be recorded during the study [ARF]:

- animal identification,
- animal gender, age and weight,
- animal health and condition,

- date and duration of surgery,
- name of the surgeon and other operators,
- description of the procedure in case of deviation to the study protocol,
- any adverse events, therapy and outcome,
- actual date of sacrifice or death.

For each surgery an individual ARF is provided to record the appropriate data.

The amendments (planned changes) and deviations (unplanned changes) to the study plan will be added to the study protocol and distributed by the PI to all parties involved in the study.

A veterinarian should always be involved in establishing, reviewing and overseeing the records.

All study data, including the ARFs, the list of participants, the randomization list, the protocol, the raw data, the reports, and all the ancillary documents will be archived at the surgical facility for 20 years.

All documents will be digitalized and saved in a dedicated databank.

13.3.11 References

They shall be critically selected and precisely focus on the topic of the trial. Consequently the list of cited publications is presented in alphabetical order and shall contain a maximum of 30 references.

13.3.12 Table of recapitulation

This is very useful to visualize rapidly the study design, and should contain the:


- number and type(s) of animal,
- number and type(s) of defect or implant/animal,
- test group(s),
- positive control group,
- negative control group,
- end point(s),
- methods of evaluation and analysis.

13.4 Choice of model

13.4.1 General guidelines

The choice of animal model should not be guided by a dogmatic way of thinking, or habit, or availability of animals, but by an argued scientific rationale founded on evidence-based preclinical and clinical data and critical

Table 13.2 Place of translational models

| Animal model | Experimental model | From pre-translational to translational aspects |
|--------------|-------------------------------|---|
| Healthy | Without oral lesion or defect |  |
| | With oral lesion or defect | |
| Compromised | Without oral lesion or defect | |
| | With oral lesion or defect | |

analysis of the international literature. This choice must incorporate the best knowledge in comparative animal anatomy, physiology, and medicine.

With respect to the framework of the present chapter dealing with large animals, it is critical that the animal model does accommodate translational study objectives.

For this purpose an updated classification of Hau, developed from his earlier one (Hau, 2003), should guide the researcher towards a final decision. It is recommended to categorize the animal–disease model in one of the following five groups: (1) induced, (2) spontaneous, (3) gene-modified (a: transgenic, b. knock-out, c. chemically induced), (4) negative, (5) orphan.

In the presently addressed field of reflexion, it is important to complement the classical notion of animal–disease model (also called animal-compromised model) with one taking into consideration and incorporating for implant dentistry the notion of bone bed and defect that corresponds to the experimental model (see the subsection entitled ‘Choice of an animal and/or experimental model’).

The resulting matrix of confronting a simplified form of the classification of Hau with the definitions of animal model and experimental model and integrating the translational aspects looks as in Table 13.2.

Furthermore the reader establish these points before definitively qualifying an animal for survival surgery in implant dentistry: (1) ethics, (2) genetics, (3) transport, housing and related animal behavior, (4) anaesthesia procedure and cardio-vascular stability, (5) bone anatomy and structure, (6) jaw and mouth anatomy, (7) alimentation and chewing process, (8) periodontium and soft tissues histology, (9) oral hygiene, (10) type of defect: induced (acute or chronic) or spontaneously occurring.

13.4.2 Swine, dog and non-human primate as animal models in implant dentistry

According to the allocated focus of the present chapter, the animal models will be restricted to swine, dog and non-human primate.

Over 20 years a large base of knowledge has been accumulated with respect to osseointegration of dental implants in large animals, and five recent reviews comparing different models by Auer *et al.* (2007), Pearce *et al.* (2007), Pellegrini *et al.* (2008), Bagi *et al.* (2011) and Dard (2012) have settled the framework and balanced the use of translational models, trying to define the relative place of each inside an experimental research framework.

Swine constitutes one of the major animal species used in translational research, surgical models, and procedural training and is increasingly being recruited as an alternative to the dog or monkey in biomedical research (Bode *et al.*, 2010; McAnulty *et al.*, 2011; Swindle *et al.*, 2011).

The testing of dental implant performance, as defined in Section 13.2, requires the recruitment of fully dentally mature animals. Adult miniature pigs generally weigh about 40 kg and consequently are easily handled, anesthetized and housed as compared to domestic farm pigs, which become much bigger with age.

Since the early 1990s (Hönig and Merten, 1993) the minipig has established itself as a standard for studies on bone regeneration in an area planned for, or treated with, dental implants (Buser *et al.*, 2004; Gottlow *et al.*, 2010; Schliephake *et al.*, 2010; Stadlinger *et al.*, 2010; Elian *et al.*, 2011).

The *dog* presents a natural susceptibility to accumulate biofilm and suffer from periodontitis, and it became consequently in the early 1960s the model of choice for research in periodontal surgery (Hennet, 1999). An extensive research is being carried out on dogs to find materials and signaling substances (growth factors, peptides) positively affecting the periodontal healing mechanism, which could turn out to be of great importance in the field of implant dentistry (Kwon *et al.*, 2010).

Recent publications point out that the development of peri-implantitis under controlled conditions and its treatment should be preferably conducted in dogs (Zitzmann *et al.*, 2004; Albouy *et al.*, 2008, 2009). It becomes important to encourage the establishment of standardized animal research procedures for that purpose.

The *non-human primate* is considered as the most accurate and reliable model in implant dentistry research. However, there are clear ethical implications in using this species.

Due to the expensive handling costs there is a temptation to maximize the number of test and control defects in a relatively small number of animals, which necessitates a careful statistical power calculation and analysis.

The vigilance of the investigators in fulfilling ethical considerations and regulations, in order to prevent any trafficking of protected species or dispersion of zoonoses and parasitic viral infections, must be high (Struillou *et al.*, 2010).

Although the non-human primate species offers a wide range of sizes, anatomical and biological features, as well as tooth size, healing characteristics, mandible kinetics and chewing abilities, closely resemble those of

humans, making this animal, in particular the baboon (Miller *et al.*, 1995), a model exceptionally suitable in dental research.

As a summary:

- the minipig is the ideal model for bone regeneration studies around dental implants when placed in intraoral situations;
- the dog should be preferentially recruited for studies conducted under compromised oral conditions (biofilm);
- the non-human primate particularly the baboon is a confirmation model which should be reserved to studies on dental implants loading.

13.5 Statistical power calculation and analysis

Dr Leticia Grize (University of Basel, Switzerland) was the main contributor to this section. This section examines the input of the statistician in preclinical performance studies outlining the elements that should be taken into consideration.

13.5.1 Adequate use of statistics

Since animal trials are experiments, they are subject to the basic principles of experimentation, such as randomization, replication, and control of variability. They should yield reproducible results, which should be appropriately interpreted. The use of statistics and the collaboration of a statistician play an important role in meeting the above requirements for the success of an animal study.

The collaboration of a statistician should start once the aim of the study is stated and the hypothesis formulated. The statistician should participate in the design and conduct of the study, and not only in the analysis of the resulting data.

13.5.2 Design of the trial

It is important to design the trial so that any observed difference among the treatment(s) and control(s) can be attributed to a real effect of treatment(s). There are several designs that can be used in an animal study (Armitage *et al.*, 2002). The most often used designs in implant dentistry animal studies are the split-unit design and the fractional factorial design.

13.5.3 Power and sample size determination

The power of a study is its ability to prove that a treatment effect exists or that the study will yield results that are statistically significant. The power

is determined by factors such as the magnitude of the treatment effect, the sample size, and the required level of statistical significance (Borenstein *et al.*, 2001). The process of power analysis is the process of finding an appropriate balance among the above three factors.

Research trials are always carried out on a sample of finite size. The results obtained in the sample are assumed to be representative of the larger population if the random sampling error is taken into consideration.

13.5.4 Importance of randomization

Random allocation of experimental units helps to avoid bias in selection that may lead to inherent differences among treatments, and provides a basis for the standard methods of statistical analysis (Pocock, 1983). Experimental units should be randomized even in the cases of split-unit and fractional factorial designs where treatments are applied to experimental subunits or when different combinations of treatments are applied to the units.

In most types of non-human experiments, randomization can usually be implemented only with minor inconvenience, since all experimental units are available at once and a tight control on how the experiment is conducted can be maintained.

13.5.5 Data recording and measurement accuracy

Once it is decided which would be the primary, secondary and tertiary variables and which are the factors to be measured, data should be recorded in well-designed forms (ARF).

Measurements should be precise, reproducible (by other observers) and independent of the observer. In addition, for the measurements to be unbiased, the treatments applied to the measured units should be blind to the observer (Kirkwood, 1989), but this is almost impossible since operators see which type of implant they insert and in which site or defect.

13.5.6 Data management and control

Animal trials are usually of a manageable size, rendering data control easier. The collected data values should be plausible, and the reason for the existence of outlier values should be examined for plausibility or possible errors.

Before statistical analysis, the data needs to be arranged and presented in a suitable form (tables).

13.5.7 Analysis of data

Analysis starts with descriptive statistics, which give a feeling for the data and express the basic results, comparing treatments in a comprehensible manner. A good practice is to start the analysis by plotting the data. Histograms and box plots can be used to examine the distribution of the data, to determine the appropriate analysis methods. Scatter plots show the relationship between factors and outcomes, and help to decide the type of relationship to be modeled in the final analysis. Data outcomes are usually summarized using means and standard deviations, and in cases of a skewed distribution using medians and quartiles.

Then, relationships between the outcome and treatment types, and between outcomes and each of the factors, that are thought to influence the outcome can be calculated. Sample size and distribution of the data determine if parametric or non-parametric statistical methods should be used to calculate those relationships.

The relationship between outcome and treatment should be adjusted at least by the effect of the known factors. In animal studies, these factors could be the animal itself, the place and side where treatments are applied, measurement methods, method of treatment application, etc. These adjustments are done using regression models. Several regression models are available. Generalized linear models provide not only the analysis of variance but also the effect size adjusted by the considered factors. Mixed models allow the analysis of replicated measurements and inclusion of random effects. Mixed models provide the flexibility of modeling not only the means of the data but variances and covariances as well.

After a model is fitted to the data, the given results of the modeling provide different statistics to be used to draw statistical inferences, test hypotheses and calculate confidence intervals.

13.5.8 Reporting study results

Tables or graphs showing the descriptive statistics of the obtained outcomes for the different treatments should be given. Reporting of descriptive statistics usually allows comparison of similar studies.

Results of the estimated effects adjusted for different factors or covariates provide the best calculated estimate of the treatment effect. These results can also be given in a graphical or tabular form and are definitely not redundant after presentation of descriptive statistics, nor do they make redundant the presentation of descriptive statistics.

It is important that the contents of tables and graphs include the estimate of the magnitude of the treatments effect, and not only the significance

level of the comparison. The significance level depends on the sample size. The statistical significance level can be given using p-values or confidence intervals.

13.5.9 Interpretation of the results

Results are to be interpreted by the scientific team as a whole. The objectivity of the statistician, due to lack of clinical involvement and mathematical training, should ensure correct interpretation of the trial findings.

The researchers performing the study (mainly clinicians) have the experience to interpret or translate the magnitude of the treatment effects to clinical effects. The size of the effect given as a number has a different meaning in different surgical situations. In addition, statistical significance is not the same as clinical importance.

13.6 Analysis

An approach associating convergent analytic methods should contribute more in the near future to increase our knowledge on the performance of dental implants.

13.6.1 Clinical observations and measurements

The assessment of the soft tissues all along the healing process and at termination, above and around the dental implants, is founded on the same clinical observations and measurements as for humans. All the investigations are conducted under sedation and with the use of a periodontal probe and possibly help of a parallel confocal scanning camera. Furthermore, fully documented cases always require related sets of photographs.

Data on the following parameters should give a good view on the soft tissues situation: free gingiva width, attached gingival width, peri-implant pocket depth (Lindhe *et al.*, 2008).

The recording of the dental plaque index and the 'bleeding on probing' test are also of interest for completeness of clinical observations (Wilson and Magnusson, 2003).

A soft tissue dehiscence grading recently proposed by Freilich *et al.* (2011) could reveal useful data in case of submerged dental implantation.

13.6.2 Parallel confocal imaging

Parallel confocal laser scanning is a system developed for dental impression that utilizes laser and optical scanning to digitally capture the

surface and contours of the tooth and gingiva in order to fit crowns and bridges (Henkel, 2007). The equipment is now available for dental practices, and consists of a handheld scanning camera and software for data processing.

Beside the above use, parallel confocal laser scanning can be applied in experimental surgical research for the quantitative assessment, at surgery and at re-entry, of soft tissues and bone shape and dimensions at defects or around dental implants. For that purpose a few technical adaptations as size reduction and handiness of the camera are still necessary. It must be noted that the large amount of gathered electronic data imposes a dedicated analysis platform and a consuming working time.

13.6.3 Regular radiology, computed tomography, cone beam computed tomography and micro-computed tomography

The historical and *regular radiology* is the basement to follow up on mineralized bone growth and resorption. It delivers two-dimensional pictures that identify material or bone loss and the occurrence of lacunae around implants, and consequently helps in the understanding of healing and regeneration.

A digital system combining fast image recovery and easy data management (Parks and Williamson, 2002) should be preferred to a regular one (Woodward, 2009).

Different regular X-ray options are possible, including intraoral, periapical, or occlusal, and extraoral panoramic or tomographic (Reddy and Wang, 1999).

The measurements and analysis must be performed and double checked by blinded investigators.

At least one study has aimed to identify radiographic methods revealing data that are the most representative of the peri-implant bone as assessed by histology in minipigs (Corpas *et al.*, 2011).

Beside the still very valuable two-dimensional X-ray analysis, some other very reliable imaging methods have been developed and deliver three-dimensional images.

CT finds its place for investigations on small and large animals.

In dentistry, preoperative tomography is usually performed before insertion of implants and delivers information about local anatomy, bone quality and volume (Eckerdal and Kvint, 1986; Andersson and Svartz, 1988). Postoperatively, bone resorption, loss of integration, bony pockets, and mechanical failures are detectable (Naitoh *et al.*, 2010; Granström and Gröndahl, 2011).

Cone beam computerized tomography (CBCT) of the craniofacial complex provides the possibility of evaluating animal anatomy, including the occlusion and tooth angulations, in three dimensions and with high accuracy (Gray, 2010; Al-Ekrish and Ekram, 2011).

CBCT differs from regular computerized tomography by higher resolution, shorter scanning times and lower X-ray exposure.

Bone and soft tissue structures around dental implants can also be examined by *micro-computerized tomography* (μ CT). The analysis of peri-implant tissues is normally done by means of light microscopy on thin histological sections. This destructive technique allows only for a limited number of two-dimensional sections on retrieved implants. μ CT is non-destructive, fast, high resolution and presents a fully three-dimensional characterization of the bone structure and volume and tissues inside the defined region of interest. The bone volume/total volume ratio (BV/TV) is the investigation parameter of reference for this imaging method.

Van Oosterwyck *et al.* (2000) claimed that the μ CT can be compared to traditional histology when examining peri-implant structures. Since then, this method has been used in a few other large animal based dental implant examinations where blocks of tissues have to be retrieved (Shalabi *et al.*, 2006). Hildebrand *et al.* (1999) measured three-dimensionally samples with a μ CT scanner and subsequently evaluated them with histomorphometry. The results showed significant differences between the methods and in their relation to the bone volume fraction.

Shouten *et al.* (2009) compared μ CT and histology at two different implants in a goat femur study and concluded that these two methods complemented each other.

Liu *et al.* (2011) insisted on the validation tests being performed for any investigation using metal implants and limiting the risks of artefacts due to the composition and geometry of the devices.

μ CT can also be applied to regenerative materials (Kon *et al.*, 2009) but the differentiation of radiological density between the substitute material and the growing bone still remains a challenge.

μ CT can be used *in vivo*, without the necessity of samples excision, but only if full-body small animals (mouse, rat) are involved. This presents the advantage of specific time intervals assessments on the same rodent (De Smet *et al.*, 2006). (See Fig. 13.4.)

It should be pointed out that imaging methods by allowing the follow-up of the same animal through several time-points can easily contribute to decreasing the number of specimens recruited in a study in the near future. Although this definitively represents an ethical and statistical added value for trials conducted on rodents, suitable equipment still needs to be developed for large animals.



13.4 Micro-computed tomography 3D reconstruction of newly grown bone at the surface of a dental implant.

13.6.4 Biomechanical analysis

The biomechanical analysis (removal torque, push out, pull out) implies an *ex-vivo* approach to be conducted immediately after animal termination on rapidly excised and non-dehydrated bone samples.

Both descriptive and functional testing of the bone-implant interface should include histomorphometry and biomechanical testing, such as torque removal values and push out/pull out strength (Cochran, 1999). It has to be remembered that the assessment of the implant stability quotient (ISQ), as recorded in human clinics, represents a non-validated method for experimental surgical research.

The biomechanical analysis finds its full sense when normal size dental implants are used in large animal performance models.

The assessment of the *ISQ* has gained an increased popularity as a non-invasive test of implant stability at implant placement, over the course of the osseointegration period and prior to prosthetic loading in humans (Bornstein *et al.*, 2009). The scale ranges from 1 to 100 and is measured by instruments using the Resonance Frequency Analysis (RFA) principle. The acceptable stability range lies between 55 and 85 ISQ. Since the

first examination in humans (Meredith *et al.*, 1997), experimental studies correlating ISQ and bone-to-implant contact (BIC) have shown divergent results (Sennerby and Meredith, 2008). The assessment of the ISQ obtained from the measurements of resonance frequency still remains only an indicative parameter non-validated for systematic animal surgical research.

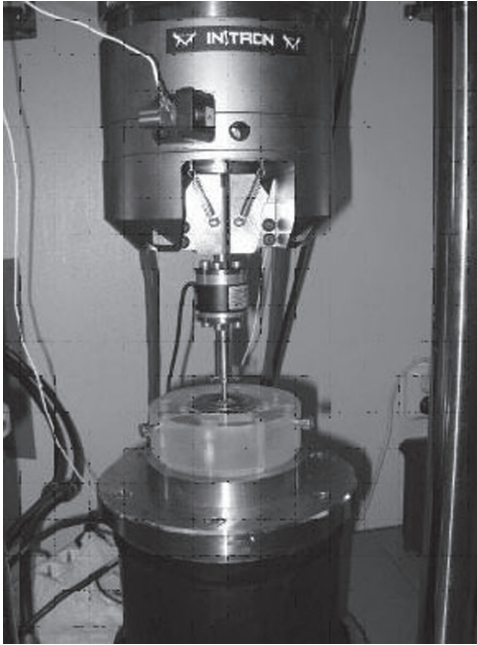
Taking place immediately after sacrifice, the *removal torque* analysis is commonly used for the biomechanical evaluation of osseointegration. The functional capacity of osseointegrated dental implants to bear load is largely dependent on the quality of the interface between the bone and the implant. The main animal model used for the removal torque analysis is the rabbit and most commonly implants are placed in tibiae or femurs (Johansson and Albrektsson, 1987). This type of examination method has since been improved and extended to large animals. Ferguson *et al.* (2006) were able to calculate precisely the bone-implant interfacial stiffness from the torque-rotation curve delivered by implants inserted in the upper jaw of minipigs. This method has shown that rough surface titanium implants are harder to remove than smooth surface implants (Javed *et al.*, 2011).

In a review by Wennerberg and Albrektsson (2009) dealing with surface topographies, different analysis methods were presented, among others removal torque and push out/pull out tests. The authors found that the surface topography influenced the bone response. They recommended an adequate standardization of measurement and evaluation techniques.

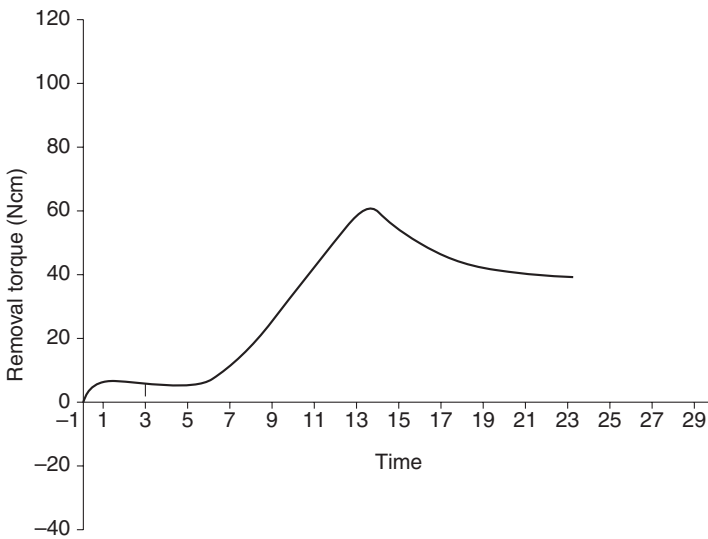
The behavior of dental implants made from different materials, such as titanium and zirconia (Gahlert *et al.*, 2010), or titanium and titanium–zirconium alloy (Gottlow *et al.*, 2010), can also be tested with removal torque (Figs 13.5 and 13.6). In both studies, the new material was at least equivalent to titanium when implanted in minipigs jaws.

Monjo *et al.* (2008) further developed a surgical approach initially established by Ronold and Ellingsen (2002) and maximized the experimental data collection after cortical positioning of disk samples on the rabbit tibia by studying the association of *pull out* tests with gene expression and volumetric bone mineral density of sub-implant cortical bone obtained by micro-computed tomography. There is speculation whether the lessons drawn from these investigations can be applied in the near future to performance study in large animals.

Nanoindentation appears to be a promising technique (Hoffler *et al.*, 2005) and can usefully complement histological analysis, because it is performed on the same histological sections without adding any specific preparation. The region of interest (ROI) sites are selected under a light microscope, which is part of the equipment, and the spot regions are randomly indented. The elastic modulus of the bone is calculated from the contact stiffness



13.5 Removal torque equipment.



13.6 Removal torque curve.

during unloading after application of a maximum load. The hardness is calculated as a function of the maximum force divided by the projected contact area at maximum load (Hoffler *et al.*, 2000; Butz *et al.*, 2006). Odgaard (1997) wrote that a full characterization of elastic mechanical properties associating three-dimensional reconstruction with newly developed methods for large-scale finite element analysis allows calculations of all elastic properties at the cancellous bone continuum level.

13.6.5 Histology–histomorphometry

As this topic is extensively treated in another chapter, the following sections will outline only specific issues related to dental implant research.

Light microscopy

The preparation of the histological sections must follow the procedure presented by Donath and Breuner (1982). This historical publication set up the still fully valid gold standards for embedding and sectioning of non-decalcified hard-tissue samples.

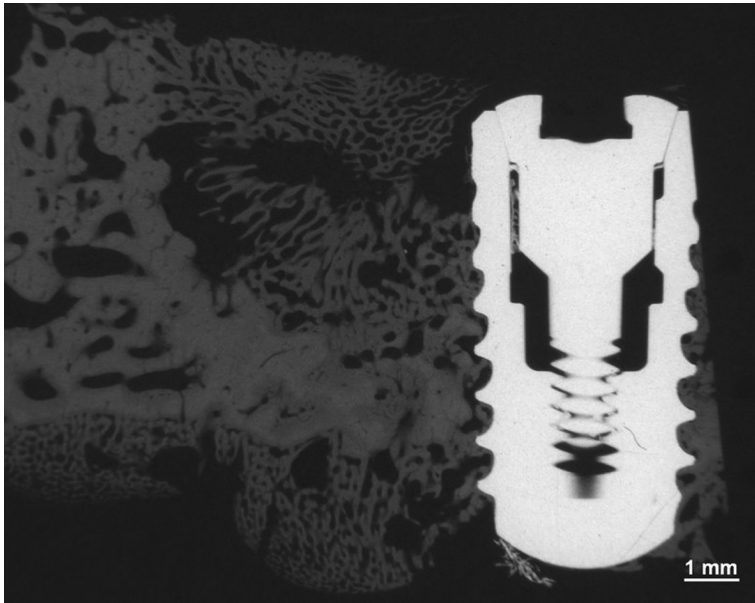
It can only be added that as a first step after sawing and grinding to a thickness of about 100 μm , an analysis based on *contact microX-rays* allows histomorphometric measurements of the mineralized tissues surrounding the implant including the bone-implant interface (see Fig. 13.7). As a second step, after reaching a section thickness of about 30 μm , qualitative and quantitative observations after *chemical staining* can be conducted.

The most critical part takes place before the observation phase by establishing the limits of the ROI. A standard approach is proposed in Fig. 13.9 later.

As a complement to the use of chemical dyes a place should be reserved for the preparation of the samples by immunohistochemical treatments. Following an embedding procedure, which is different from the one reported above and which requires some attention, *immunohistochemistry* techniques open wide perspectives for hard and soft tissues observations.

Arvidson *et al.* (1996) examined immunological and vascularisation markers in soft tissues adjacent to two different dental implants. Haga *et al.* (2009), Hou *et al.* (2009) and Kim *et al.* (2007) aimed at detailing the process of implant osseointegration or surrounding tissues remodeling in rats.

The lack of dyes addressing antigen–antibody reaction in large animals gravely impairs the implementation of immunohistochemical observations, particularly in dogs and human primates. Decisive advances applying to minipig research are currently happening in this domain (McAnulty *et al.*, 2011).



13.7 Contact micro-X-rays of a dental implant surrounded by native (bottom) and newly grown bone (top).

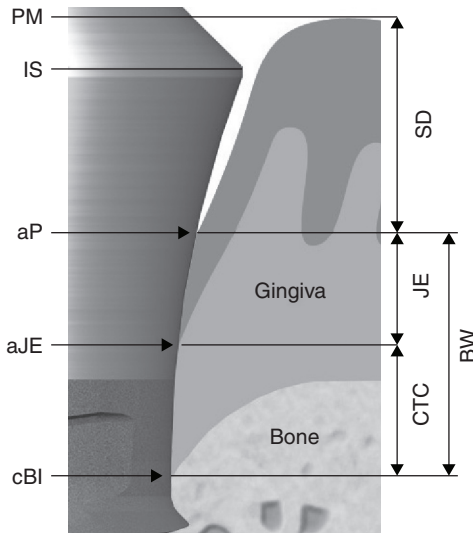
Soft tissues

Expertise still needs to be developed to fully understand the critical aspects of the morphology and the physiology of these tissues, which guarantee the future biological integration and functionality of a dental implant.

The following morphological parameters should be the subject of quantitative assessment:

- abutment height,
- gingival sulcus height,
- junctional epithelium length,
- connective tissue contact length,
- soft tissue contact length or biological width.

As the gingiva are particularly sensitive to the sample harvesting and surgical handling, which induce a local exsanguination and consequently changes in the shape and size of the gingival sulcus before the full fixation process is completed, we do not recommend considering the gingival sulcus height in the calculation of the biological width to avoid measurements bias. This is in disagreement with the definition of Laney (2007), which assumes a full-body animal fixation



13.8 Histological landmarks for soft tissues. PM: peri-implant mucosa (soft tissue margin); IS: implant shoulder; aP: apical limit of pocket or sulcus; aJE: apical limit of junctional epithelium; cBI: coronal bone-implant contact; SD: sulcus depth; JE: junctional epithelium; CTC: connective tissue contact; BW: biological width.

procedure before samples harvesting. This type of fixation is difficult to set up in the majority of the experimental surgical research facilities.

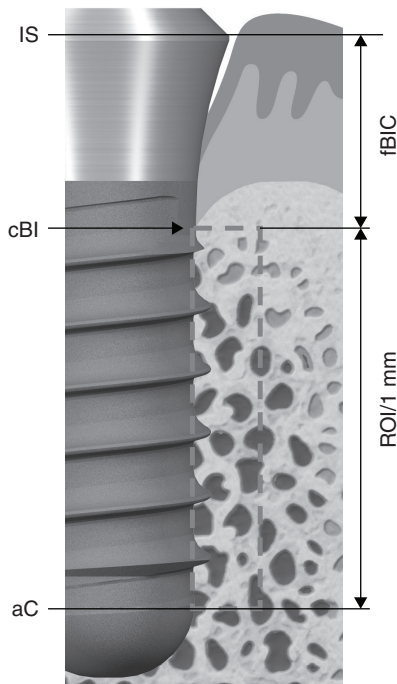
We propose to restrain the definition of the biological width to the addition of the junctional epithelium and connective tissue lengths. If we suppose that these two elements form a ‘sealing zone’ around the dental implant structures they should be less sensitive to the retrieval process and likely to be more representative of the soft tissues integration. (See Fig. 13.8.)

- collagen I fibers orientation inside the connective tissue.

Bone

The morphological parameters that are under quantitative assessment are well known and can be listed as follows:

- fBIC: first bone-to-implant contact;
- BIC: bone-to-implant contact;
- BA/TA: bone area on total area which corresponds to the bone density inside the ROI. The ROI extends from the cBI (coronal bone-implant contact) to the aC (apical limit of cylindrical part) and its width is commonly 1 mm on each side of the sectioned implant. (See Fig. 13.9.)



13.9 Histological landmarks for bone. IS: implant shoulder; cBI: coronal bone–implant contact; aC: apical limit of cylindrical part.

Scanning and transmission electron microscopy

Scanning electron microscopy (SEM) allows for the detection of changes in surface structures of dental implants and on peri-implant soft or hard tissues after harvesting (Sennerby *et al.*, 1989). This observation method relies on a fast samples preparation and easy observations and could efficiently complement the biomechanical analysis.

Li (1997) observed remaining tissues and newly formed bone on implant surfaces after push out test. Wennerberg and Albrektsson (2009), performing implant surfaces characterization, incorporated SEM analysis in their approach. They showed that surface topography did influence the osseointegrative outcomes and that measurement and evaluation techniques need to be standardized.

Gedrange *et al.* (2009) used a pig mandible model to compare different implants after immediate or delayed loading.

Wierzechos *et al.* (2008) opened the possibility of studying the cells at the implant-bone interface in pig mandibles with an SEM/backscattered electron imaging method.

Transmission electron microscopy (TEM) provides high resolution images of tissue, bone and implant surfaces. These ultrastructural examinations focused on cells and structures at the implant soft- or hard-tissue interfaces (Stefflik *et al.*, 1984, 1990).

TEM can be used to analyze the leakage of titanium fragments from implants into the surrounding tissues. Although Tanaka *et al.* (2000) identified titanium leakage in the mandible of beagle dogs, this detection approach was not conclusive in a study in monkeys (Lee *et al.*, 2009).

According to Shimizu-Ishiura *et al.* (2002) the use of TEM could be appropriate when implants are coated with organic or non-organic molecular materials.

It is accepted that TEM requires special specimen preparations, based on ultrathin sections, and does not allow observation of a dental implant and its surrounding tissue on all surfaces. Consequently TEM does not represent a first choice for studies on implants, but can usefully complement other methods.

13.6.6 Gene expression analysis

Although very high performing, and giving insights into initial events of biological integration, this approach is still not very common, possibly due to the fact that the interpretation of up- or down-regulated genes is difficult to relate to implant integration.

Testing of bone formation/resorption and inflammatory genes can, however, be very beneficial to understanding the implant behavior towards hard and soft tissue.

The harvesting of the samples, the subsequent RNA extractions and DNA duplications must be thoroughly conducted under controlled conditions.

The final analysis will be performed by microarrays or Polymerase Chain Reaction (PCR) techniques.

The microarrays method could be superior to the single gene expression testing, since all genes are assessed at once and a genes map can be drawn and genes regulation can be compared.

Although microarrays methods are promising per se as a comparison method for different implants, they have only been employed in a few studies, and mainly to test implant biocompatibility.

Bone healing around implants was tested by Kojima *et al.* (2008). Mueller *et al.* (2010) compared different implants and implant insertion techniques.

More commonly and preferably, a few well-chosen genes are tested in one study. Bone matrix-genes were assessed by Yamano *et al.* (2010). Bone matrix proteins, such as bone sialoprotein, osteopontin, and osteocalcin were also the target of gene expression profiling on titanium implants possessing a new, nano-treated surface (Mendonça *et al.*, 2009).

Increased of bone matrix or osteoblast-related genes is often interpreted as a proof of osseointegration but this method should not be used alone but in combination with other much classical approaches.

13.7 Translation from animal studies to human clinical trials

The translation, or more correctly the translatability, corresponds to the degree of correlation between preclinical study outcomes and clinical situations in humans. It refers to the clinical predictability.

The primary goal of translational research is to integrate advancements in molecular biology with clinical trials, taking research from the ‘bench-to-bedside’ (Woolf, 2008; Goldblatt and Lee, 2010). This approach is a two-direction communication pathway, from scientists to clinicians and from clinicians to scientists.

Experimental surgical research in animals is fully part of this process. The first advancement to be accomplished consists in the validation of the animal and the experimental models. This chapter signals a first attempt in this direction. A new treatment for a particular condition developed in animals should normally end up with a clinical success in human trials. This conclusive step in humans constitutes the ultimate validation, and opens the use of an evidence-based device to the broader patient population.

As seen throughout this chapter, a considerable experience in designing and executing animal trials, from the proof-of-concept stage to the pivotal preclinical study for regulatory submission, is mandatory.

The extensive review by Perel *et al.* (2007) clearly illustrated this challenge, presenting examples taken from different medical fields.

Furthermore Sah and Ratcliffe (2010), reporting on NIH panel general consensus, claimed that there is a substantial need for improved and standardized animal models for tissue engineering and regenerative medicine of the musculoskeletal system, and that animal models, especially large animal models, are critical to the preclinical step of translating research from bench-to-bedside.

We fully agree with this position, and consider and recommend that in the future fewer animal studies will be conducted, but they will be much better integrated inside a coherent research program covering the full properties of a dental implant. Each study will be probably larger, implementing ‘all in one’ regular (i.e. histology) and more sophisticated methods of investigation (i.e. genes expression analysis).

13.8 Acknowledgments

The author thanks Ms Beata Fabi for her support during the critical screening of the literature, Dr Leticia Grize for writing the section on statistics and

Mr Marcel Obrecht for the photographs and for regular very stimulating discussions on several topics of this chapter.

13.9 Sources of further information and advice

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13.9.2 Norms

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Non-clinical functional evaluation of medical devices: general recommendations and examples for soft tissue implants

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Abstract: Non-clinical functional studies are often needed to evaluate performance as well as the safety of a medical device under conditions of use. This chapter describes how to design a compliant functional study based on available standards, illustrated through examples dealing with the functional evaluation of soft tissue implants. The choice of the appropriate animal model, mimicking the intended clinical use will be explained. The definition of the objectives of the study based on the product characteristics, the use of appropriate evaluation tools, the definition of appropriate control groups and time-periods will also be discussed. Finally, strategies to incorporate requirements of other safety standards in the design of combined functional studies will be described.

Key words: animal model, functional study, safety, performance, study design, soft tissue implants.

14.1 Introduction and definitions

Performance of a medical device can be defined as its ability to accomplish its intended purpose in contributing to the treatment of a disease.^{1,2} Performance can be measured against standards of accuracy, completeness, and speed. The performance of a medical device can include both its technical and biological performance.

- Technical performance is the ability of a device to accomplish expected technical functions in clinical use. These functions may include ease of handling, the proper functioning of an alarm, or a variety of mechanical properties. Technical performance is evaluated mainly through bench testing, however some of these characteristics can also be evaluated during non-clinical *in vivo* studies under simulated conditions of use.
- Biological performance is the ability of a device to produce the intended biological effect relative to the targeted medical conditions without causing adverse effects, and is evaluated through non-clinical studies.

In vitro studies can be used when an appropriate model is available to demonstrate a specific performance endpoint (e.g. release of an antimicrobial agent from a coating). However, non-clinical performance is in general assessed by testing the product in a situation mimicking as closely as possible the final use of the product, that is, through implantation in an *in vivo* model in what is commonly called a (non-clinical) functional study.

Functional studies are generally designed to evaluate performance, but also to evaluate safety, under conditions of use. Safety can be defined as the absence of unreasonable risk of illness or injury with the use of the device for its intended purpose. During the development phase, the medical-device manufacturer should demonstrate that all possible risks associated with the use of the device have been identified. In some cases performance is closely linked to safety, as a lack of performance can involve safety issues. For example, a monitor alarm that does not perform well (technical performance issue) or restenosis of a stent after implantation (biological performance issue) could pose serious clinical safety problems for the patient.

As an example to illustrate these definitions, consider an endovascular stent.³⁻⁶

- Technical performance involves the handling characteristics of the delivery system and characteristics of the stent at implantation (e.g. ability to access the target site and withdraw, visibility, deployment of the stent, stent fixation in the vascular wall, and others). In this case, technical performance is extensively evaluated through bench testing, and some properties are also evaluated under *in vivo* conditions simulating clinical use during functional testing.
- Biological performance involves the level of restenosis of the arterial lumen after implantation as compared to an already approved stent. This is assessed through angiographic and histomorphometrical measurements of the diameter of an appropriately sized artery after a period of stent implantation. The coronary arteries of the pig may be used for such studies. In this case, lack of performance can be responsible for loss of arterial patency, therefore causing safety issues.
- Safety parameters include evaluation of local biological responses at the implanted site (thrombosis, inflammation, lack of endothelialization, necrosis, edge effects, and restenosis as described above), as well as at the heart downstream from the stent (stent-related pathology) and at the systemic level (thromboembolisms at the level of the organs perfused by the stented vessel).

14.2 The purpose of functional studies

The purpose of functional studies may differ according to the product's stage of development (see Table 14.1). At early stages of development, preliminary

Table 14.1 Functional studies for implants

| | Preliminary study | Main study |
|----------------------|--|---|
| Device | Development stage | Final device |
| Design | Acute/short-term study | Longer term study up to steady state |
| | One time point | Several time points |
| | Limited number of implanted sites | High number of implanted sites |
| Objective | Screening | Performance |
| | Proof of concept | Safety |
| | Evaluation of the main biological issues | Establish requirement for transition to clinical trials |
| | Verify the suitability of the model | |
| Study quality status | – | GLP |

functional studies can be used to select candidates of different prototypes permitting selection of the best candidate, or helping in the improvement of design. Preliminary functional studies can also serve to demonstrate proof of concept and to evaluate the main biological issues. Finally, preliminary functional studies can be very helpful in verifying the suitability of the model and endpoints selected for evaluation, and in refining a protocol for the main functional study. At this stage, the study can be a short term evaluation including a limited number of implantation sites. When the device reaches its final stage of development, the purpose of a functional study is to show with reasonable probability that the product intended for treating a specific condition will be effective, and that the product will not compromise the clinical condition of patients or the safety of users. These data may also be used to establish requirements to be included in the human clinical study design. The main functional study for a long-term implant will generally include several time points, with an appropriate number of tested sites per time point (see Section 4.5).

The quality status of the studies, as well as the quality status of the contract testing laboratory itself, should be carefully evaluated. The contract testing laboratory should be selected based on its scientific experience and its quality status (laboratory accredited in compliance with the standard ISO 17025,⁷ Good Laboratory Practice (GLP) approval⁸), ensuring good quality control and reliability of the results of all studies conducted in the laboratory. For preliminary studies, the GLP status of the study is not needed nor recommended to provide the needed flexibility. The GLP status is recommended for main functional studies including assessment of safety, in particular if a product file is likely to be submitted to the US Food and Drug Administration (FDA) (and this will probably also be a requirement of other authorities soon). Finally, it is best to use the same contract testing laboratory for both phases (preliminary and main study) to take advantage of the ‘learning curve’.

14.3 Standards and documentation

No standard dealing with functional evaluation of safety and performance is available. If a standard or guideline exists for a given device category, this can give some indications about the model or design to be used. However, these documents never provide exact details of the protocol to be used. In some cases the provisions of a standard are deliberately written without detail, so as not to inhibit innovation. When dealing with innovative devices, often no standard is directly applicable. Therefore, manufacturers can develop their own strategies to demonstrate conformity of their device to the relevant essential principles of safety and performance, provided that the design and selected model are clearly justified.

A functional study always needs a customized protocol, tailored to meet the scientific and regulatory questions raised by each new product. Study design should be based on expected performance, existing standards or guidelines if any, risk analysis, and principles of good science. The design also depends on the data available for component materials, the device, the route of administration, or the targeted indication. In addition, the combination of different test methods is possible and recommended when feasible (see Section 14.5): in this case the functional study should be carefully designed to cover the requirements of the corresponding combined standards.

No standardized protocols for functional studies are available. However, various documents (as listed in Table 14.2) can help in the design of a functional study. Some information can be found in standards or guidance

Table 14.2 Initial documentation for the design of a functional study

| Document | Web link |
|-------------------------------------|---|
| FDA guidance document | http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm |
| EMA guidance document | http://www.ema.europa.eu |
| ISO standards | http://www.iso.org/iso/iso_catalogue.htm |
| ASTM | http://www.astm.org/Standard/index.shtml |
| Summary of safety and effectiveness | http://www.fda.gov + keywords |
| Literature data | http://www.ncbi.nlm.nih.gov |
| List of recognized standards: | |
| – by FDA | http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm |
| – by Europe | http://ec.europa.eu/enterprise/policies/european-standards/documents/harmonised-standards-legislation/list-references/medical-devices/index_en.htm |

documents dealing with a similar category of device (ISO standards, FDA or EMEA guidance documents, or national standards such as ASTM). Some interesting information about the strategy of testing can also be gleaned from the 'summary of safety and effectiveness' of a similar device that has already been submitted to the FDA and approved. This information is published on the FDA website once a product receives Pre-Market Approval (PMA). Finally, literature dealing with evaluation of similar devices, or describing the anatomic and physiological bases of the targeted organ or function in different species, is of great help when designing appropriate functional studies.

14.4 How to design a functional study

A customized design will usually be defined and justified for each functional study according to the product characteristics and what needs to be demonstrated.

When designing an *in vivo* functional study, a list of general questions should be addressed:

- What is the intended use of the product, and does the model mimic the intended clinical use?
- Are the objectives of the study clear?
- What laboratory animal should be used and why?
- What controls are appropriate?
- What are appropriate time periods and overall length of study?
- What is the appropriate number of tested sites?
- How can the design of the study be written to incorporate requirements of other standards? (Section 14.5)

Responses to these questions will be illustrated through some examples dealing with the functional evaluation of soft tissue implants.

14.4.1 What is the intended use of the product, and does the model mimic the intended clinical use?

The intended use of the product should be clearly defined in order to choose the appropriate surgical model. The implantation site should reproduce the complex biological interactions between the device and the tissues receiving the implant. A route of administration and surgical procedure should be selected that simulates in the animal the proposed clinical use. For example, when studying a device intended to be used in abdominal surgery, the route

of administration (laparotomy versus celioscopy) should be known. One intended use should correspond to one study; if one study is used to cover several intended uses, this should be clearly justified.

In some cases, it is not possible to mimic exactly the intended clinical use. For example, anatomic features and the use of a non-diseased animal may make it difficult to evaluate a heart valve substitute implanted by minimally invasive techniques in positions for which it is intended (orthotopic position). In this case the ISO 5840-3 standard⁹ authorizes the use of an alternative site, or the use of alternative implantation procedures, if a justification is provided. Indeed, currently there is no ideal animal for cardiac valve evaluation.¹⁰ The healthy sheep is preferred for *in vivo* assessment of aortic valve devices and can provide validation of several features. However sheep do have several limitations such as (1) the small size of the femoral arteries through which such devices are inserted; (2) angulation of the aortic arch (cause of kinking of the delivery system); (3) the length of the aortic arch (shorter than in humans); (4) the location of coronary ostia (closer to the aortic valve than in humans, which can cause myocardial infarction at implantation); (5) the lack of calcification and stenosis of the native valve, which can cause anchoring difficulties of the device. Therefore, with justification, this model may be modified, for example by placing the valve in a heterotopic position (e.g. at the level of the descending aorta after creation of a native valve insufficiency) instead of orthotopic (at the level of the native valve).¹¹

14.4.2 Are the objectives of the study clear?

Study objectives should be clearly established before beginning the study. Primary and secondary objectives – generally including safety and performance objectives – should be identified based on standard requirements, risk analysis, product specificities and mechanisms of action. Clear objectives allow the definition of the appropriate endpoints in the selected animal (often different from the endpoints of a clinical study).

In most cases, the objective of a functional study is to demonstrate, on the basis of performance, the equivalence or superiority of a new device compared to a marketed device without compromising safety. Because the range of differences observed can be minimal, the demonstration of superiority can be particularly challenging for incremental improvements of performance. To enable objective comparisons when evaluating products, the use of quantitative endpoints is recommended whenever possible. The number of sites implanted should be sufficient to allow statistical comparisons. If quantitative measurements are not possible, one should define a system for semi-quantitative scoring of parameters permitting a comparison of the different products tested. For example, use of histological analysis of

the implanted tissue is often needed to describe the implant/tissue interface. However, histological analysis should be not only descriptive, but should also include a system for semi-quantitative scoring of selected relevant end-points, and quantitative histomorphometrical measurements may be used when possible.

14.4.3 What laboratory animal should be used and why?

To ensure that an animal for a functional study is appropriate, there are several considerations:

- Ethics: the laboratory chosen to perform the study should comply with national regulations for the health and welfare of experimental animals. Then the selection of the animal and model should take into account the three R's rule (Reduce–Replace–Refine) including the following points: Reduce the number of animals being used, Replace animal models when possible and Refine the methodology used.
- The anatomy and size of the targeted site should mimic as closely as possible the clinical situation. For some studies, small animals (rat, rabbit) can be selected. However the size of the animal, certain physiological differences, or lack of ability to mimic the human surgical procedure, can lead to limitations when extrapolating to the clinic. Therefore, large animals are generally preferred even though these models also involve inherent limitations.
- The physiology and metabolism of the selected species should be known, in order to ensure that it is representative of what will be encountered in clinical use. Moreover, the availability of specific blood or histochemical markers in selected species should be investigated if they are needed.¹²
- The age of the animal is a critical element, as it can influence the data obtained. When performing long-term studies, non-growing animals should be selected. Pigs have been a well-recognized experimental animal in biomedical research for many years because of the morphological and physiological similarities between porcine and human organs, especially the skin, cardiovascular system, gastrointestinal tract, and urinary system. However, the weight of a domestic pig at 3 months is around 50 kg, whereas 6 months later it reaches 150 kg, to attain 250 kg in adulthood. Thus juvenile pigs are preferred for handling reasons, whereas adult domestic pigs are rarely used. However, it is not recommended to use juvenile domestic pigs for studies longer than 1 month. Indeed, as an example, if a coronary stent is implanted in a 3-month-old domestic pig for 6 months, the results obtained will be completely distorted as the implanted site grows with the animal. For long-term studies, preference

should be given to the minipig that is smaller and lighter, can be used at full maturity, does not grow during the course of the study, and is easy to handle. In contrast, evaluation of an anticalcification treatment of a cardiac valve (calcification being a drawback of the biological valves over the long term) requires implantation in a juvenile animal model, known to be more prone to calcification, permitting evaluation of the calcification after a short time period.¹³

- In theory, the use of pathological models is ideal, since they are supposed to mimic closely the clinical situation. However, the model can be difficult to standardize, and the pathology induced can differ from the clinical and may be associated with high morbidity and mortality. Therefore the pros and cons of such pathological models should be evaluated carefully before use. Several pathological models have been described and can be used in functional studies. The use of such models is particularly useful when the device targets a specific pathological condition that is well simulated in the model. Pathological models typically used include: diabetic models (chemical or surgical induction by pancreatectomy in several species, rats and mice transgenic diabetic strains), osteoporotic models (induced in several species by ovariectomy of female animals combined with a low calcium regimen and/or steroid treatment), atherosclerotic models (induced by a high cholesterol regimen in several species), or infectious models (different models available according to the location of the infection, inoculated strain and concentration among others).
- The medical treatment of animals during the testing period is also very important. The treatment used should correspond to the state-of-the-art treatment mimicking the clinical situation. However, attention should be focused on the specificities of the metabolism of the animal species, which may require some adaptation in the medical treatment. For example, a *per os* treatment given to a sheep shows an absorption profile after administration completely different from that of humans because of differences in digestion. Therefore, in cases such as these, the treatment must be adjusted in order to find the appropriate dose and/or mode of administration to cause the appropriate pharmacological activity.

The evaluation of wound healing is a good example to illustrate the difficulties of selecting a suitable model. Wound healing is a complex physiological process, including several distinct steps and involving different biological reactions.^{14,15} Numerous animal models of wound healing have been described, from mice to pig.^{16–20} These models can be used to evaluate the performance of treatments on acute or chronic wounds, on detersion, granulation, epidermization, or prevention of infection. Several models with different specificities have been described; however, investigators

must remember that no model is an exact replication of the clinical condition. Despite thousands of publications using mice for wound-healing experiments, there are many differences between the healing of these small rodents and that of man. All rodents have a muscle below the derma called *panculus carnosus*, which does not exist in man (except in the temporal facial area), and which is responsible for major wound contraction when it is altered during creation of the experimental wound, thus limiting the evaluation of a true wound healing process through granulation and epidermization. In mice, it is difficult to avoid alteration of this muscle during wound creation, therefore the use of mice should be avoided unless a specific transgenic strain is needed: in this case techniques preventing contraction of the wound should be employed.^{20,21} In the rat and the guinea pig, even if this *panculus carnosus* exists, it is possible to create wounds without modifying this muscle, using microsurgery techniques, therefore allowing the use of these animals in wound-healing studies without this contraction factor interfering with interpretation.^{18,22} The advantages of the rat model are its availability and cost, ease of handling, and the ability to withstand frequent dressing changes without anesthesia. However, some differences exist between rodents and humans regarding skin anatomy and physiology, such as their dense layer of body hair, thinness of the dermis and epidermis, minimal dermal papillae, absence of pigments, absence of apocrine and eccrine glands, and presence of *panculus carnosus*. Anatomically and physiologically, pig skin is more similar to human, and has been shown to be an excellent model for the study of wound healing.^{19,23} The structure, composition, size, and healing process are very similar in human and pigs, facilitating the study of different phases of healing and treatment. Functional evaluation of large active systems, such as those used for negative pressure wound therapy, can also be tested in the pig. The drawback of this model is the cost, housing, and handling of these large animals, as well as the necessity of anesthesia at each dressing change.

In healthy young animals, healing occurs very rapidly and efficiently, which can decrease the possibility of differentiating between treatments based on accelerated healing. Therefore in some cases it may be necessary to use models of impaired healing. Factors that can impair healing include malnutrition, infection, ischemia, diabetes, venous stasis, pressure, treatments with steroids, chemotherapy agents, or radiation. Models that address these problems have been developed and are described in the literature.¹⁷ They can be helpful to demonstrate the superiority of a treatment under specific conditions; however, it is important to remember that all models have their limitations and no model will completely represent the human situation.

Finally, some factors in the design of these wound-healing studies strongly influence the results and should therefore be carefully addressed. These include the standardization of the wounding technique (surgical,

burn, chemical, etc.), position of the wounds, frequency of dressing changes (which should mimic clinical treatment), secondary dressing (occlusive, semi-occlusive, gauze or others), wound washes and moistening, standardization of the macroscopic scoring system and measurements (granulation tissue and epidermization), and histological techniques (including immunohistochemistry, evaluation of cell proliferation and collagen maturity). This illustrates the fact that the evaluation of a quite simple device such as a dressing can be a complex challenge at the stage of design of a functional study.

14.4.4 What controls are appropriate?

The design of a functional study should always be comparative. It should include a reference group that corresponds to the state-of-the-art treatment, when this exists and/or of a similar product with the same indication already cleared on the market of the target country ('substantially equivalent predicate device'). If possible, the selected control implant should have physical characteristics and mode of action similar to those of test implants in order to permit appropriate comparisons. If no equivalent exists, then the comparison is based on the existing treatment for the indication. The choice of the reference treatment is crucial, as it can strongly impact the conclusion of the study. Therefore it is recommended to collect a maximum amount of information about the possible reference treatments before finalizing study design. The inclusion of operated non-implanted animals ('sham') in the design can also be very helpful when discriminating the effects of the surgical procedure by itself and when characterizing the biological response observed in the selected model without treatment. Although it may not appear useful at the stage of study design, the inclusion of reference and/or sham groups should not be omitted as they can be very helpful if a negative observation is made after implantation. Indeed a comparison of the observations from the different groups can help to exclude the incrimination of the tested implant.

For functional testing of combination products (device plus drug), additional points should be taken into consideration. The primary mode of action of the combination product should be demonstrated. Then one should consider the intended therapeutic effect as well as potential local and systemic side effects of each constituent separately. Finally, one should take into account the duration of action of each constituent part toward the therapeutic effect of the combination product. Consequently, when dealing with a combination product, the study design should always include the evaluation of each constituent, both separately and combined, in order to differentiate between the effects of each constituent at different time points (see Section 14.3.5).

This may be illustrated using the example of a drug eluting stent (DES), which consists of a stent coated with an antiproliferative drug. The objective of this combination is to reduce cell proliferation and subsequent restenosis of the vessel lumen after implantation. The animals recommended in the evaluation of DES include the domestic pig and the minipig. The coronary arteries of pigs after injury are known to respond in a similar fashion to human coronary arteries.¹⁰ There is no doubt that the arteries in non-atherosclerotic animals cannot fully imitate the human condition, however, non-clinical animal studies still have predictive value because the biological processes associated with arterial repair are similar. Study design for the evaluation of this type of device is described in guidances from the FDA and EMEA.^{3,4} These guidances require the evaluation of the different components both separately and in combination: stent alone, stent + coating without drug, drug alone and DES (stent + coating + drug). In addition to these groups, an already marketed predicate DES is generally tested in order to demonstrate the equivalence or superiority of the new DES versus the predicate. The performance – defined here by the restenosis reduction of the implanted artery after a defined period of implantation – is evaluated using standardized measurement tools such as quantitative angiography and histomorphometry. The results from the different groups are compared in order to illustrate the effect of each constituent part towards performance (anti-restenosis effect) and safety.

The time periods (see Section 14.4.5) for evaluation of a combination product should be carefully selected in order to demonstrate the contribution of the drug (action and duration) versus the device without the drug, and the absence of side effects related to each component. In most cases, the drug component has a short duration but the device and/or the coating used as a drug carrier may have a long one. Therefore, at least one short and one long time period should be included. The short one evaluates early drug and/or carrier effects, and the long one assesses the delayed effects. For example, for DES, the FDA and EMEA guidances recommend both a short (1 month) and a long time period (minimum 6 months) to evaluate the long-term performance but also delayed adverse effects that can be caused, for example, by the degradation of the carrier covering the stent struts.

14.4.5 What are appropriate time periods and overall length of study?

The choice of time periods is critical in the design of a functional study. It should be adequate to demonstrate the equivalence or superiority of the tested product as compared to the control(s). Sometimes, the time periods recommended are described in the vertical standards or guidances; for

example, ISO 7198, dealing with evaluation of vascular grafts, recommends functional implantation of the graft for a minimum of 20 weeks in a minimum of six animals. The time periods should be chosen based on the objectives of performance knowledge of the implant characteristics and risk analysis. The selected species (large or small) influences the length of these studies, as the life spans of small and large animal species vary greatly. Therefore the choice of the time periods also depends on the animal model and the physiopathology of the targeted tissue or function in the selected species. For long-term implants, at least two time periods are generally included: the first one is used to evaluate how the device performs and how the surrounding tissues react early after implantation; the second time period corresponds to the biological steady state after implantation (from a performance and safety point of view). Additional intermediate time periods before steady state occurs may also be useful to differentiate between the performances of two products or to demonstrate a mechanism of action.

In the case of a degradable implant, an additional issue is an understanding of the functionality of the implant *in vivo* during the course of degradation, and its safety in the presence of degradation products in surrounding tissues. In general, the design of the studies dealing with degradable implants includes at least three time periods, which are based on knowledge of the material and its degradation kinetics:

- The first time period addresses the very beginning of degradation (in general 2–4 weeks following implantation depending on implant characteristics).
- The second time period allows observation of reactions during degradation of the material.
- The third time period should correspond to a steady state with ideally nearly complete degradation of the material and full restoration of tissue integrity. When the *in vivo* degradation process is not fully understood, it is recommended to keep an additional satellite group running longer, in case degradation is still not complete in the last group observed.

14.4.6 What is the appropriate number of tested sites?

As previously discussed, the evaluated endpoints of a study should be quantitative or semi-quantitative in order to allow objective comparisons, including statistics if possible. Sometimes the number of tested sites to be included in a functional study is described in standards or guidances, however this information is rarely available. Existing data from the literature or from preliminary studies can be used to determine the number of sites to be included. The required number depends on the intrinsic variability

among the animals being used, the consistency of the surgical procedure, the accuracy of the evaluation methods, and the statistical techniques used to analyze the data. Where no data are available, the use of a minimum of ten sites per group and per time period is generally recommended, allowing the use of reliable statistical tests. Increasing the number of sites per group improves the power of the statistical comparisons. If the study design does not include enough sites and no statistical analysis is proposed, this should be justified as its validity could be criticized. Finally, the addition of spare animals – to be used to replace any lost during the course of the study – is strongly recommended, in order to follow the initial design if unexpected deaths are observed. The number of tested sites that should be included can also be driven by the requirements of other combined standards, as described in the following section.

14.5 Combining non-clinical functional studies with requirements of safety standards

Functional study design can be adapted so as to cover the requirements of other standards and thus avoid study replication. For example, it can be adapted to cover the requirements of some parts of the ISO 10993 horizontal standard, which describes the evaluation of the biocompatibility of medical devices. Among them, the most common standard to be combined with functional studies is ISO 10993 Part 6,²⁴ describing the evaluation of local effects after implantation.

ISO 10993 Part 6 describes how to evaluate the effects of the device on the implanted tissue, as well as the effect of the tissue on the device. This evaluation is based on macroscopical and histological analyses and comparison with a control implant. ISO 10993 Part 6 describes standardized sites for implantation such as subcutaneous, intramuscular or intraosseous based on the intended use. However, when possible, it is recommended to evaluate local effects after implantation in tissues like those encountered clinically. The evaluation of the local effects after implantation can therefore be part of the safety parameters of the functional study.

The type of animal described in ISO 10993 Part 6 is varied, ranging from mice to sheep. The standard recommends the use of animals of a similar age, gender, and strain, and the use of similar implantation sites. Larger animals are generally used for longer time periods. According to the standard, the time period can range from 1 to 104 weeks; typically the design should include a short time period (1–12 weeks) in order to evaluate the early effects and one long-term time period (> 12 weeks) that should correspond to the clinical time of exposure or to a biological steady state.

If the product is degradable, as described in Section 14.4.5, a minimum of 3 time points should be included (before, during and at the end of the

degradation period). If *in vivo* degradation kinetics are not known, a pilot study is recommended to help define the appropriate time periods. It is also recommended that a satellite group be kept longer in case the degradation observed in the last group is not achieved. If the product is degradable, attention should be given to the location of the implanted site as it can be very difficult to identify it at termination; the aim is to ensure appropriate histological analysis of the tissues at the implant site. Finally, the standard also recommends analyzing the draining lymph node in order to detect any toxic effect of the degradation product in these filtering tissues.

The number of sites required by ISO 10993 Part 6 is a minimum of ten per product and per time period, with a minimum of three animals; moreover if possible, the test and the control items should be implanted in the same animal, in order to decrease inter-animal variability. Study design should always be comparative and should include a negative control (known to induce a minimal reaction), or a control article that corresponds to a device with established biocompatibility used clinically and preferably with similar physical characteristics (size, form and surface), as such characteristics can influence local tissue reaction.

Evaluation of local tissue effects is primarily based on semi-quantitative histopathology of 12 tissue responses, scored from 0 to 4 and a comparison of the averages obtained with the test and control implants, to obtain an Irritant Ranking Score (IRS). Based on the IRS, the test article is considered as non-irritant, slightly, moderately, or severely irritant. Then the significance of the result is assessed and related to the intended application (a slight irritant may be acceptable for certain applications) and on the kinetics of response over time especially in the case of degradable products.

14.6 Conclusion

Although great emphasis is often placed on the importance of clinical data, non-clinical data, based upon valid scientific methods, are critical to demonstrate device safety and performance. Non-clinical data may significantly influence risk/benefit determinations in premarket review.²⁵ Indeed, medical devices often have attributes that cannot be tested using clinical methods alone but that play a major role in safety or performance. For example, information such as reactions at the implant/tissue interface or biomechanical behavior after implantation cannot be obtained from patients.

Functional studies should be carefully designed and conducted to provide appropriate specific information enabling device characterization and providing valid scientific evidence supporting its safety and performance. The development, use and interpretation of data from animals can be a complex challenge, requiring multidisciplinary skills such as surgery, animal

Table 14.3 Key points to combine a functional study with the requirements of ISO 10993-Part 6 (Evaluation of the local effects after implantation)

| Design | Requirements of ISO 10993-6 |
|--|--|
| Animal model | From mice to sheep; animals of a similar age, gender, strain |
| Implantation site | Standardized, in contact with tissues simulating the clinical use |
| Comparative control | Negative control and/or control device used clinically with established biocompatibility and approaching physical characteristics |
| Time periods | If possible, implant test and control in the same animal One or two time periods, selected according to the clinical use and expected biological reaction: <ul style="list-style-type: none"> – One short time period: between 1 and 12 weeks (acute effects) – One long time period: more than 12 weeks (steady state or end of the clinical exposure) |
| Number of sites | Minimum of ten sites per product and per time period in a minimum of three animals Inclusion of spare animals recommended |
| <i>Additional requirements for degradable products</i> | |
| Implantation site | Appropriate site location at implantation to find out the sites at termination |
| Time periods | Three time periods: <ul style="list-style-type: none"> – One before the beginning of the degradation – One during the degradation – One at the end of the degradation or steady state |
| Number of sites | Addition of a satellite group strongly recommended in case degradation is not achieved in the last group |
| Other | Sampling of the local draining lymph nodes |

care, histology, study supervision and regulatory management. Therefore, the choice of the contract testing laboratory that performs a functional study must be based on the experience of the laboratory in these fields, as well as on available resources, ethical approval, proposed time lines and regulatory compliance.

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Mechanical testing for soft and hard tissue implants

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Abstract: Despite the growing knowledge of loads acting on implantable devices, mechanical failure is still present in clinical practice. The root problem is a dramatic underestimation of the complexity of interactions between implant design, materials, and the biological system. This chapter summarizes the biomechanical basis of implant performance testing and its application to selected types of implants. In addition, it also provides a critical review of the limitations of test results.

Key words: implant, mechanical testing, *in vivo* loads, implant safety, failure.

15.1 Introduction

The primary objective of biocompatibility testing is to examine the effects of the complex biochemical interaction between the implant materials and the patient. In comparison to the biochemical system, the biomechanical loading conditions are relatively straightforward. In fact, engineers started to investigate the human locomotion system in the nineteenth century (Wolff, 1892) and discovered the interaction between external loading of the skeleton and bone growth.

The first biomechanical models applied simple static approaches. Since then, the advancement of mathematical methods and tools has helped researchers understand the dynamic nature of motion (Andersen, 2010). Most recently, implants equipped with sensors have measured the *in vivo* loading during activities of daily living (Bergmann, 2010).

Despite the growing knowledge of loads acting on implants, mechanical failure is still present in clinical practice (Atwood, 2010). There are a number of reasons for this, all of them stemming from one basic problem: a dramatic underestimation of the complexity of the interactions between the implant design and materials and the biological system. Implants are subjected to aggressive chemical environmental conditions and complex multidirectional loads, yet are expected to provide lifelong service with no possibility of routine maintenance.

This chapter will summarize the biomechanical basis of implant performance testing and its application to selected types of implants. The first section will

provide an overview of the methods used to perform mechanical testing. The subsequent sections describe specific implant groups, providing a brief summary of the state of the art in mechanical testing accompanied by a critical review of the limitations of the test results. Future trends in mechanical performance testing and recommended readings are provided in Sections 15.5 and 15.6.

15.2 Principles of setting up a mechanical test

The design of a mechanical test series is of fundamental importance to ensure its overall success. Establishing the correct mechanical loading conditions and test environment is essential to guarantee test relevance and obtain meaningful results; improper selection of test parameters will render the effort worthless. Likewise, defining acceptance criteria for assessing implant performance is straightforward for well-known systems or predicate devices studies. However, establishing such criteria when testing novel implant designs (i.e. designs for which there are no comparable previous studies) can present a serious challenge and requires careful consideration.

15.2.1 Loading conditions

Test standards that describe the loading conditions of implants were developed based on a consensus between nominated experts worldwide. Each standard reflects the knowledge and the design philosophy of implants at the time of its writing. However, the *in vivo* conditions change gradually, driven by younger, more active patients and an increasing average patients' weight. Therefore, a new design principle that is introduced may need to exceed the test standard. For example, *in vivo* fractures occur in modular hip stems that initially passed fatigue tests described by ISO 7206-4 and ISO 7206-6 (Grupp, 2010).

To reduce time and costs, most test standards describe benchmark testing rather than more complex loading regimes based on a complete biomechanical approach. Early failure of an implant with a well proven successful clinical history can occur when new information about the loads acting on the implant is combined with existing standard test protocols. In the past this has led to arbitrary criticism of test standards.

Standard types of implants benefit from the profound knowledge summarized in test standards and from direct comparison to historical data from predicate devices. When new designs or material features are introduced, the state of the art *in vitro* tests must be critically reviewed for applicability. Fortunately, there are three sources that can be used to define test parameters for innovative implants.

First, the strictly biomechanical approach used intensively in past decades may be sufficient to calculate the forces and moments acting on an implant. The principles of technical mechanics can be used to develop simple

mechanical models that may underestimate the real loading conditions. Real loads are typically enhanced by the action of agonist and antagonist muscle groups. Software packages such as AnyBody® (AnyBody Technology A/S, Aalborg, Denmark) or SIMM (MusculoGraphics, Inc., Santa Rosa, USA) include models of soft tissues and the dynamics of motion and may be used to generate more realistic test data.

Second, reliable data have recently become available through *in vivo* measurements using instrumented joint prostheses. A large database including different types of implants is freely accessible at www.orthoload.com. Data from a large number of patients are already available for standard implants such as total hip replacements. Other types of joint replacements are currently being investigated to collect data from a statistically relevant number of patients (Westerhoff, 2009).

Finally, data may be available from tests of a predicate device. These data may include clinical experience and therefore taps the most valuable source of information, the patient. This source of data is limited to new implants with only minor innovations and must be combined with custom test procedures or modifications of existing test standards to evaluate the specific design features of the new product.

Almost any loading condition can be duplicated in the test laboratory. The financial resources of the project often limit the test engineers' ability to implement complex loading regimes. A reduction of the loads and moments acting on an implant will reduce test costs dramatically. Uniaxial testing is therefore used for most test procedures. Fortunately, uniaxial loading does not necessarily result in the generation of simple stress conditions within the implant. Complex interactions between shear and normal stresses can be achieved through the careful selection of the boundary conditions in the test frame.

The second approach used to lower test costs and time is to increase the test loading frequency. The upper limit is a function of the test standard and of the performance of the test frame and implant material. Some metal components can be tested at 30 Hz test frequency; implants made of plastics may require frequencies of 2 Hz or even lower. To investigate the effects of corrosion, the test frequency must be reduced to 1 Hz for most tests (Goldberg, 1997).

For all testing procedures described above it is assumed that the simulation is performed using sinusoidal loading conditions. This loading pattern is well established for standard fatigue testing and has been proven to replicate clinical failure for most available implants. Recent studies have shown that some failure mechanisms are triggered by single events or activities of daily living that are not covered by the standard load cycles designed to simulate typical activities such as level walking (Park, 2006; Bal, 2008; Saito, 2008; Thomas, 2008). Therefore, the introduction of 'heavy duty cycles' has become a major task for research and standardization. Fortunately, most modern test systems are computer controlled and can easily handle complex load regimes.

15.2.2 Test environment

A considerable number of mechanical tests are performed in conditions that are dry and at laboratory room temperature. This procedure is less expensive, faster and allows the specimens to be inspected without interrupting the test. The major limitations of this procedure are the absence of corrosion effects that might occur *in vivo* under wet conditions and temperature effects that might change the material behavior of the implant. Fatigue testing of a metal bone plate is typically performed dry at room temperature, whereas fatigue testing of bone cement requires a water bath heated to 37°C. It is important to note that a surrounding water bath enhances any test artifacts resulting from self-heating of the specimens during dynamic loading. The water bath acts as a thermal resistance, increasing the specimen temperature. One solution for this problem is to externally cool the water bath.

Distilled water is typically used for tests in which the temperature of the specimen is of interest. The use of sodium chloride at 9.0 g/L results in a better replication of the *in vivo* conditions. Sodium chloride baths are used to examine corrosion effects such as fretting corrosion of modular connections and stress corrosion that reduces the fatigue properties of the device. Fatigue testing is typically performed at high test frequencies in order to reduce test times and costs. However, the repassivation time is several milliseconds, meaning that corrosion tests require low-cycle frequencies to replicate *in vivo* conditions (Schaaff, 2006). For this reason, any corrosion occurring during high-frequency fatigue testing should be regarded with great care. The absence of corrosion in standard fatigue tests does not conclusively show that the implant has been successfully tested for corrosion effects. More aggressive test fluids, such as Hank's or Tani-Zucci solutions, should be used instead. Nevertheless, it is difficult to replicate *in vivo* corrosion effects that occur in the patient over the course of decades (Grupp, 2010).

For tribological and tribochemical applications the use of solutions containing proteins is advised. From the different types of serums available, newborn calf serum or bovine serum are considered to be the standards for implant wear testing. It has been found that the protein concentration of the test fluid has an impact on the test results (Wang, 2004). It is therefore good practice to use a defined protein concentration of 20 or 30 g/L. This approach is preferred over that of older test standards, where a dilution of the initial serum is described. The interaction between the test fluid and the articulating surfaces is still under investigation and will require intensive research in the future (Dwivedi, 2009).

Regardless of the type of test fluid selected, the fluid may diffuse into the material of the test specimen (Roba, 2009). Fluid absorption can change the material properties, geometry, and weight of the implant. This may influence calculations of wear that is based on the weight loss of the specimen. From

Table 15.1 Test frame comparison

| Test frame | Load | | | Frequency | Price |
|-----------------|--------------|---------------|--------------|-----------|-------|
| | Small (mN–N) | Medium (N–kN) | High (kN–MN) | | |
| Hydraulic | o | ++ | ++ | ++ | o |
| Pneumatic | – | ++ | + | + | + |
| Electromechanic | + | ++ | + | – | o |
| Moving magnet | ++ | + | – | ++ | o |

++ = Excellent, + = good, o = acceptable, – = not acceptable.

a mechanical point of view, lubrication of modular implants can reduce the stability of the connections. Testing of constructs such as spinal-implant systems may therefore result in lower mechanical strength when performed under wet conditions.

15.2.3 Test frames

Four different types of test frame are available: hydraulic, pneumatic, electromechanical, and moving magnet (see Table 15.1). In the past, the specific characteristics of these frames have limited their usage. However, modern test equipment can be used for a wide range of biomechanical applications. There are still specific advantages that may aid in the selection of the best in-house system or contract test lab.

Test frames driven by an electric motor are mostly used for quasi-static applications. This type of frame is standard equipment for almost any biomechanics lab and can perform all single-cycle or low-cycle fatigue tests. Electromechanical systems can generate extremely low test forces (mN) up to high test forces (MN).

Whenever dynamic and/or fatigue testing is required, three other types of test frame are available. Hydraulic actuators offer a high performance in terms of test frequency and system durability, and can generate medium (N) to extremely high (GN) test loads. Because the hydraulic power supply emits heat and noise, it is often located outside of the test lab. Centralized pump systems are able to supply multiple stations within one test lab, and can become cost effective for a larger number of test stations.

Pneumatic actuators are less expensive but still provide an acceptable stroke rate and durability. Again, the need for a centralized power supply might arise, due to the noise and heat generated by the pump system.

Moving magnet actuators have been used in the past for high-frequency applications. New test frames can generate low (mN) to intermediate (kN) test forces for static loads. This type of actuator is the first choice for tests requiring

extremely low test loads. These loads cannot be generated by hydraulic and pneumatic systems due to frictional effects of their internal seals.

15.2.4 Number of specimens

The sample size of the test is defined based on the lot size and the desired confidence level of the results. This calculation typically results in dozens of test specimens. Most normative references and custom test plans follow a more pragmatic approach, balancing the need for maximum implant safety with economic boundary conditions. There is a general trend towards using smaller sample sizes to test final products, while using larger sample sizes to conduct material tests of easier to manufacture, standardized samples.

For example, the fatigue testing of bone cement described in ASTM F2118 requires a minimum of 45 test specimens, whereas only six samples are required for fatigue testing of a total hip replacement stem. Most (quasi-) static tests are only performed on five samples. This method is assumed to result in acceptable standard deviations.

ASTM E739 describes the statistical analysis of fatigue tests, including the prediction and confidence intervals. Further information about statistical planning of fatigue tests can be found in Little (1975). From a mathematical point of view, the required sample size also depends on the lot size. This approach is commonly used in pharmaceuticals and mass production but has not yet become standard for orthopedic products. ISO 16269 standards describe in detail how to determine the number of samples required for a given lot size.

An excellent summary of the statistical planning for a complete test series can be found in Box Georg (2005).

15.2.5 Acceptance criteria

Only a few test standards provide acceptable performance levels. Typically, the user of the standard must follow different methods to define the acceptance criteria.

Depending on the target market, health authorities such as the Food and Drug Administration (FDA) may provide data indicating the performance value to be reached. Most guidelines are available on the homepage of the health authority (see for example www.fda.gov). As previously mentioned, new and innovative devices might not be covered by national guideline data. In these cases, performance values can be inferred by testing a predicate device that has a successful clinical history. Naturally, highly innovative and novel implants may not have a predicate device on the market.

This problem can be addressed by defining biomechanically based performance levels. This approach is very time consuming and challenging. As shown in Section 15.2.1, the biological system is highly complex and our

knowledge of the individual implant environmental conditions is limited. Based on this complex system, only a limited number of test parameters must be selected while still maintaining a high safety level. A balance of those two conflicting interests must be reached when defining biomechanically based performance levels.

15.3 Implant-specific mechanical performance testing

The following sections will provide examples of how the principles described above are applied to specific implants. To date, a complete summary describing the mechanical tests to be performed for each type of implant does not exist. Yet most biomechanical test labs operated by manufacturers and contractors are familiar with the test standards available. Umbrella standards such as ISO 21534 and the FDA guidance documents – without claiming to be complete – are useful resources when becoming familiar with the test requirements.

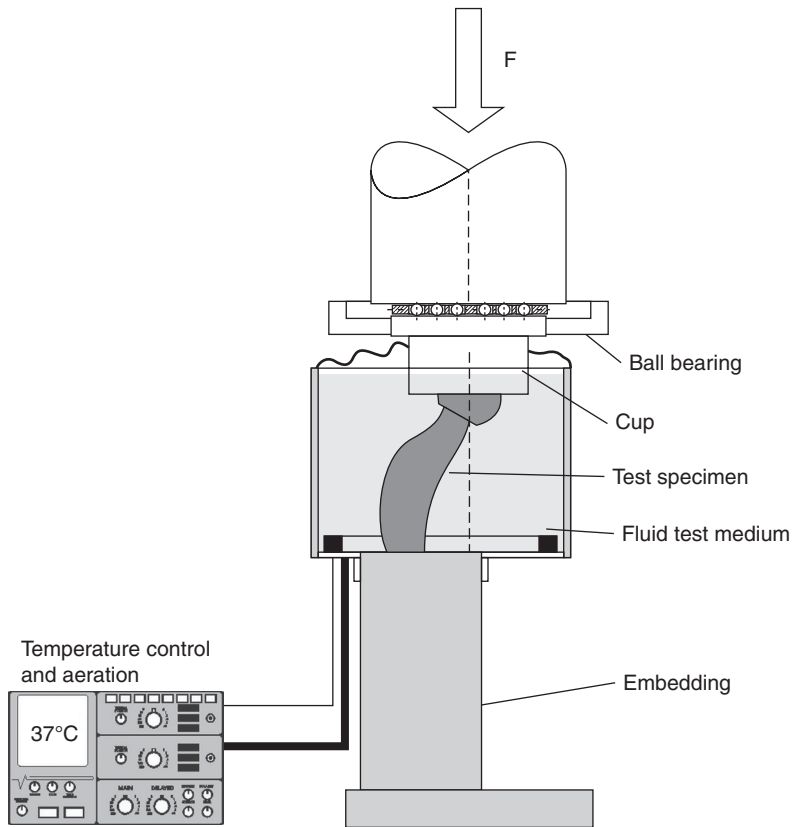
15.3.1 Fatigue testing

Implant fatigue testing is one of the most common tasks in any biomechanical laboratory. Whereas the results of static testing are typically predictable (i.e. determining implant strength), results obtained during dynamic tests (i.e. fatigue testing) can be surprising and unpredictable in nature. Likewise, fatigue limits tend to be multifactorial and may deviate considerably from static test results.

Hip stems

Clinical fracture of hip replacement stems drove the development of ISO 7206-4 in the 1980s. Analysis of retrievals indicated proximal loosening of the hip stem within the bone cement. Consequently, the resultant hip joint force was applied to the hip stem with an increased lever arm (Wroblewski, 1979). High stresses finally led to fatigue failure of the stem at approximately 80 mm below the center of the head.

The loads acting on a hip joint vary in time and depend on the type of activity. *In vitro* simulation of the complex loading pattern would require expensive multi-axis test equipment. For economic reasons and because of the limited availability of multi-axis test frames, the editors of the standards reduced the loading pattern to a single axis. Two major force directions have been identified as relevant for fatigue failure: the vertical force component creating a bending moment combined with a lateral offset of the hip stem, and the anterior–posterior force direction generating a torque moment (Fig. 15.1). Both types of loads are simulated by inclining the hip stem in the frontal and sagittal planes with respect to the test load.



15.1 Test setup used for fatigue testing of hip stems. The stem is rigidly fixed at the distal portion and the load is applied onto the modular head.

When defining the test standard, biomechanical models were used to calculate the loads acting on a hip stem. Based on those models, a test load of 3 kN was established. During laboratory tests performed using this load level, failure occurred in hip stems already proved safe by their clinical history. Following a pragmatic approach, the test load was reduced to 2.3 kN (Paul, 1999).

In addition, one million load cycles per year was established as a rule of thumb at the time the test standard was defined. A more recent publication found that 1.1 million cycles per year better represents today's younger and more active patients (Morlock, 2001). Based on a lifetime of more than 20 years, the 5 million test cycles requested by the test standard seems to underestimate the *in vivo* conditions. However, it is highly unlikely that patients walk for 5 years under the conditions described above. The extreme loosening of the hip stem during severe simulated loading conditions may therefore have to be considered carefully.

In contrast, for well-fixed hip stems the failure mode may not be loosening but rather material failure. A second test procedure is typically used for hip stem fatigue testing; ISO 7206-6 describes a method for endurance testing the neck region of a femoral stem. The ISO test standard does not indicate the test load or the number of cycles. Those values can be found in ASTM F2068 (10 million cycles, 5.34 kN load). Due to the logarithmic nature of fatigue (Schijve, 2009), increasing the number of cycles, for example, to 20 million, gives only minor additional information about the fatigue properties of the implant.

In the past, the '4 + 2' method was widely used for hip stem fatigue testing. In this method, four implants were loaded at a load level causing failure of the device and another two samples were used to confirm the run-out level at 5 and 10 million cycles, respectively. The current version of ISO 7206-4 requires six samples to be tested at a defined force level that is below the fatigue limit of most devices. This method has some intrinsic disadvantages when compared to the '4 + 2' method, which results from the difference between values (number of cycles to failure) and attributes (passed/failed).

Spinal implants

Most of the principles explained above for total hip replacements also apply to spinal-implant constructs. One additional aspect related to this implant group is the modularity of the components. A number of test standards have been developed to address this fact. The most basic standard on single components, ASTM F2193, describes tests for rods, screws, and plates. This type of test is less time consuming and therefore more economic than testing a whole implant in its clinical configuration. On the other hand, this approach is more similar to a material test than to a construct test. Consequently, the test results do not replicate *in vivo* loading, but instead must be compared to clinically successful predicate devices tested under the same conditions. This type of testing is often used to investigate the impact of design and material modifications rather than to establish a final safety certification.

Tests for partially assembled constructs are described in ASTM F1798. Modular connections such as screw-rod fixations are included in this protocol. Again, this test allows designs to be compared in a more efficient way than a complete construct test, but does not allow final conclusions to be made about the implant in its clinical configuration. This is typically done using the 'corpectomy model' described by ASTM F1717. This test applies compression-bending or torque moments to a complete construct. Currently, performance criteria are not included in the test standards. The static and fatigue properties therefore must be compared to the *in vivo* loading conditions (Westerhoff, 2009).

More flexible implant systems have been designed in the past to provide physiological loading of the spinal cord and stabilization of the vertebrae and

facet joints. From a mechanical point of view, to test those implants the test setup must provide a certain degree of axial support to the implant. This has been incorporated using spring elements (ISO 12189) and a fixed axis of rotation under displacement control (ASTM F2624). The ASTM standard focuses on 'motion preserving' implants and the ISO standard applies to 'semi-rigid' and 'flexible' designs. The distinction between 'rigid' and 'semi-rigid' implants is not defined in detail but the worst-case conditions simulated by ASTM F1717 must be applied whenever the stiffness of the implant is sufficiently high.

Additional implant specific tests are available, such as ASTM F2077 for cages and ASTM F2694 for facet joint replacements. So far, vertebral body replacements (VBR) and spinal disc implants are not covered by test standards (other than ISO 18129 for wear testing of spinal discs) but can be fatigue tested based on ASTM F2077. ASTM F2789 describes fatigue testing of nucleus implants, but the protocol was still under discussion at the time that this chapter was written.

Stents

As defined in the section on 'Hip stems', the typical number of load cycles applied to orthopedic implants ranges from 1 to 10 million. The rationale for those numbers is based on estimates of number of load cycles during daily activities. An additional test parameter for mechanical-loading tests of vascular implants is the change of blood pressure with each heartbeat, typically ranging from 80 to 130 mmHg (106.4–172.9 mbar), at a heartbeat rate of about 72 bpm (1.2 Hz). Over a 10-year *in vivo* period, the heart will beat roughly 400 million times, and hence representative mechanical testing should comprise around 400 million loading cycles. Because of this large number of required loading cycles, typical test frequencies of up to 40–90 Hz are used resulting in test times between 17 and 7 weeks, respectively. As shown in Section 2.3, this requirement is best met by electrodynamic actuators typically called 'voice coil actuators'.

To simulate the *in vivo* conditions, the stent is placed into a mock vessel using the clinical implantation procedure. The mock vessel itself must have a compliance similar to that of natural vessels, which is typically within 3.5–5.0% at 100 mmHg pressure change. An important factor for understanding the mechanical-loading conditions is that the stent is unloaded at increasing internal pressure. Hence, the use of less compliant mock vessels will therefore generate higher material stresses than a 'softer' mock vessel.

ISO 25539-2 summarizes a large number of mechanical tests for vascular stents. In addition, ASTM F2477 provides a detailed description of fatigue testing. ISO 7198 establishes test conditions to determine the dynamic compliance of vascular prosthesis and is widely used to determine the compliance of mock arteries.

While cardiovascular stents are primarily loaded by the radial motion of the artery, peripheral stents used commonly for external iliac, subclavian, renal, popliteal and femoral arteries are also loaded by bending and torsion of the soft tissue. This generates the need for more complex, multi-axis test set-ups that apply larger motions at lower test frequencies (typically 1 Hz) in bending and/or torsional direction. At the time of writing this chapter, a consensus document about the test parameter for peripheral stents does not exist.

15.3.2 Wear testing

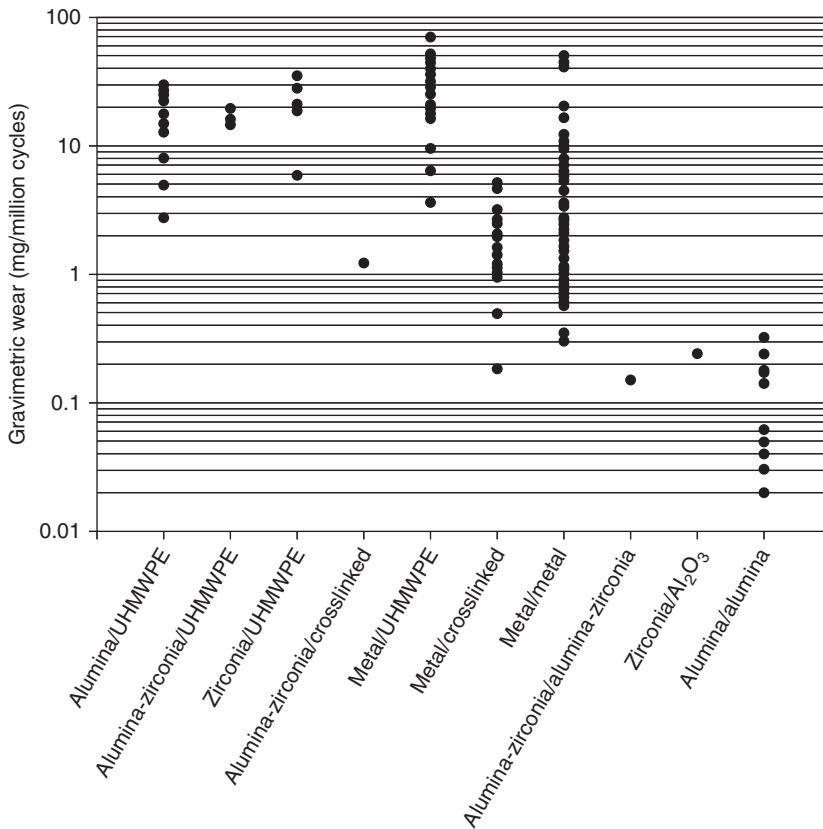
Wear testing attempts to replicate the complex interaction between mechanical, biochemical and electrochemical processes in the human body. Our current understanding of tribological processes is based on mechanical engineering concepts and has only recently been applied to the functionality of synovial joints and their associated endoprosthetic replacements.

Hip joint replacement

Wear testing of joint implants is one of the most demanding tasks in bio-mechanical science. First of all, the loading and the environmental *in vivo* conditions need to be replicated as closely as possible to mimic the implant specific wear mechanisms. The expected *in vivo* service time also needs to be simulated within an acceptable *in vitro* test period. As shown for fatigue testing of hip stems, five million test cycles corresponds to approximately 5 years of use by the patient. As known from basic tribological laws ('Stribeck curve'), the wear behavior changes with increasing relative-motion velocity at the articulating interfaces. The test frequency is therefore limited to 1 Hz (1 cycle per second) for most tests, resulting in a total wear test duration of approximately 10 weeks for a hip- or knee-replacement implant. Even this low test frequency does not replicate the resting periods of activities of daily living. In addition, environmental effects, such as oxidation of plastic materials, are not simulated.

Wear testing of hip implants has been performed since 1966 (Duff-Barclay, 1966) using different test set-ups worldwide. Today, two test principles have been implemented by test standards: ISO 14242-1 describes a wear test frame that simulates all *in vivo* motions (flexion/extension, adduction/abduction and internal/external rotation) and ISO 14242-3 covers an 'orbital bearing' test frame that uses a single axis of rotation to apply bi-directional motion at the articulating interface. An overview of the current test frames can be found in Affatato *et al.* (2008).

Typically, wear is measured by weight loss of the components (ISO 14242-2). This method has proven to be accurate within a typical precision of approximately 0.1 mg. This accuracy is sufficient for hard-on-soft articulations such as metal on polyethylene, but might be close to the wear rate of the coupling



15.2 Wear rates measured by EndoLab® for total hip replacements.

for hard-on-hard bearings, such as ceramic on ceramic (Richardson, 2005). Geometrical measurements of the worn areas are performed on explanted devices but do not offer the precision of the weight-loss method. The fluid uptake of plastic materials must be corrected by 'loaded soak controls' allowing for the differentiation of weight gain caused by absorption of the surrounding test fluid and weight loss caused by wear. This effect of fluid absorption depends on the surface temperature. Neglect of this factor has led to the publication of arguable wear data in the past (Muratoglu, 2001).

Typically, at least three specimens are tested in combination with one loaded soak control. The specimens are cleaned and weighed after 0.5 million cycles, and at 1 million-cycle intervals thereafter. Most hard-on-soft couplings show linear wear behavior. Hard-on-hard couplings tend to show higher wear rates for approximately the first 1 million cycles, followed by lower steady state wear rates thereafter. The wear rate(s) are linearized to establish the implant specific wear rate in mg per million

cycles or in mm^3 per million cycles (for better comparison to the clinical literature). As shown in Fig. 15.2, the wear rate differs even for nominally identical wear couplings. This effect can be attributed to the fact that different materials may share the same name or to differences in designs, surface properties and sterilization doses and methods. It is therefore misleading to indicate a given wear rate for a given type of implant, such as metal on polyethylene.

The test fluid is a factor that must be considered for wear testing. As described by the test standards, newborn calf serum is used at a defined protein content of 30 g/L. This value corresponds to clinical data published by Brandt *et al.* (2010). However, the serum is a mixture of proteins and a number of other organic and non-organic substances (Mazzucco, 2004). The impact of these individual ingredients on the wear rate is highly relevant for the reproducibility of the test method. Recent studies have shown that the test fluid interacts with polyethylene and with metal surfaces (Wimmer, 2010). More insight into the underlying mechanisms may lead to better predictions of the *in vivo* wear behavior of implants. This may play an important role in the effort to reduce wear of artificial joints.

To date, performance values for wear tests have not been provided by test standards or health authorities. Nevertheless, comparison to the literature allows the definition of an acceptance level.

Knee joint replacement

Most total knee replacements provide a metal-on-polyethylene articulation. Clinical problems are typically related to the wear of the polyethylene component(s). From a biomechanical point of view, motions and loads acting on a total knee replacement are more complex than for a total hip replacement. The dominating factor is the geometry of the knee joint, which offers additional degrees of freedom when compared to the ‘ball and socket’ joint of the hip. Consequently, wear testing of a total knee replacement is more complex than wear testing of a total hip replacement.

ISO 14243-1 describes the loads and motions used to simulate level walking on a total knee replacement. This standard describes how to apply the loads and moments as a function of the flexion angle of the knee replacement. While this method is mechanically stable for conforming types of knee implants, it would presumably become unstable for less constraint implants. Those implants are designed such that the soft tissue is needed to balance the loads not being transferred by the joint replacement. Test frames following the ISO 14243-1 standard typically use mechanical springs at a given stiffness to simulate the soft tissue restraint. The displacements of the implant during testing depend on the design and allow the prediction of *in vivo* motions.

Table 15.2 Comparison of ISO 14243-1 and ISO 14243-3

| Direction | ISO 14243-1 | ISO 14243-3 |
|--------------------|-------------------------|-------------------------|
| Axial | Force controlled | Force controlled |
| Anterior–posterior | Force controlled | Displacement controlled |
| Rotation | Force controlled | Displacement controlled |
| Flexion–extension | Displacement controlled | Displacement controlled |

For historical reasons, a second test standard using a different approach is available; ISO 14243-3 describes the anterior–posterior and rotational motions instead of the loads (as given by ISO 14243-1). The loads generated depend on the degree of constraint of the individual implant. A direct comparison of the two standards is provided in Table 15.2.

ISO 14243-2 describes the method used to quantify the amount of wear for knee joint replacements (corresponding to ISO 14242-2 described previously for hip joint replacements).

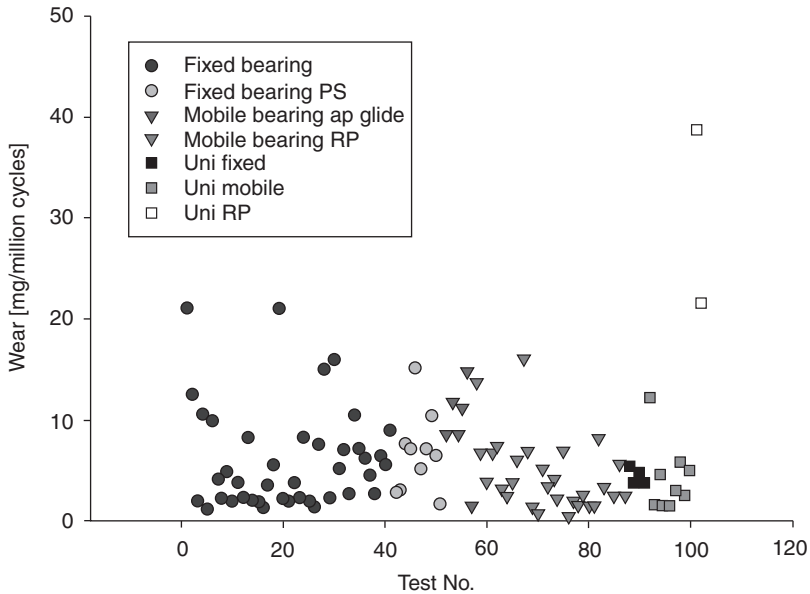
For knee and hip replacements, the total number of cycles is limited to 5 million resulting in a typical test period of about 10 weeks. In our test laboratories we have shown that the wear rate of total knee replacements is less sensitive to alterations of the test fluid than that of hip replacements. While the differences in fluid film formation and contact pressures are assumed to contribute to this behavior, further research is needed to more fully understand the underlying mechanisms.

Figure 15.3 shows a summary of wear test results obtained in our laboratories for different types of total knee replacements. Similar to total hip replacements, the individual design groups offer a large variety of wear rates. It would therefore be misleading to directly compare groups of implants rather than individual implants.

15.4 Advanced therapy products (ATPs) – cartilage

The mechanical tests used for cartilage tissue are based on material tests designed for technical applications. Typical material properties are measured in compression, tension or shear mode (Chen, 2004). Because of the biphasic nature of cartilage, additional time dependent parameters such as creep and stress relaxation can be measured (Soltz, 1998). Typical parameters are the aggregate modulus (H_A), the Poisson's ratio (ν) and the permeability (k). Certain mechanical parameters have been proven to discriminate between normal and degenerated cartilage (Katta, 2008; Brown, 2009).

The measurement of the frictional coefficient may help to characterize new developments in cartilage replacement and to gain an understanding of the factors affecting the tribological conditions of human cartilage (Katta, 2008). ASTM F2451 describes mechanical standard methods for *in vitro*



15.3 Wear rates measured by EndoLab® for different types of total knee replacements. The types of knee implants displayed are: fixed bearing, fixed bearing posterior stabilized, mobile bearing with anterior–posterior (ap) glide, mobile bearing with rotating platform, unicondylar fixed bearing, unicondylar mobile bearing and unicondylar rotating platform.

cartilage testing and refers to published data to be used as a performance standard.

Cartilage testing provides only a snapshot of a dynamic *in vivo* process and does not allow for long-term evaluation. This is despite the progress made in our understanding of the biochemical nature of the mechanical properties being measured. To investigate cartilage under simulated *in vivo* conditions, test stands allowing for short-term (Northwood, 2007) and mid-term (Wimmer, 2004) application of loads and motions have been developed. Standard analysis methods, such as live/dead assays, gene expression, and others, are performed to understand degradation effects. The major limitations of these tests relate to the complex *in vivo* biochemical environment of cartilage and to contaminations caused by the test setup. Typical test times (including resting periods) are 2–3 weeks.

Some implants are intended to be mechanically loaded within a short time after implantation, whereas others gain their biomechanical competence through the formation of a collagen matrix over the course of weeks or months. To date, a test standard simulating the growth period of this type of implant is not available.

15.5 Conclusion and future trends

Because of the complex *in vivo* loading and environmental conditions, in the past mechanical testing of implants was based on clinical failure modes observed rather than on the prediction of new failure modes by biomechanical models. One reason for this shortcoming was the lack of sophisticated test equipment, especially for wear testing of total joint replacements. Improvements in the design of test frames, especially the introduction of digital electronics, offer a variety of new options. One exciting and promising possibility is to simulate activities of daily living, such as rising from a chair, descending stairs, and performing turning maneuvers. The introduction of additional load blocks has been shown to generate more realistic wear patterns and sufficiently reproduce the clinical failure modes observed (Popoola, 2010).

In the past, best case environmental and post-operative conditions were assumed for testing. Factors such as implant alignment and third body contamination by bone cement particles are known to impact the wear results and to generate additional wear mechanisms, such as impingement or subluxation of implants. The introduction of adverse conditions is a move towards more realistic *in vitro* testing. In addition, knowledge of the sensitivity of the individual implant to suboptimal implantation will help the educated surgeon choose the best implant for the individual patient.

The interaction between the implant and the biological environment is still not well understood. Simple mechanical tests, such as bending of femoral nails, can be performed under dry laboratory room conditions, whereas more complex mechanisms, such as fatigue corrosion effects, have led to clinical failure in the past. The underlying mechanism is still not well understood, and is of great interest for future research.

Advanced therapy products, such as cartilage replacement, require the interdisciplinary effort of biomechanical, biochemical, and biological research. At the same time, methods known from biology need to be introduced to mechanical test set-ups. This is a demanding task that must be achieved to facilitate future progress in our ability to replace living tissue with living tissue rather than with materials derived from technical applications.

15.6 Sources of further information and advice

The nature of biomechanics is highly interdisciplinary. Therefore, a profound knowledge of classic mechanics as imparted in engineering science is recommended. A thorough summary of the basics and their application to orthopedics can be found in the book of Mow and Hayes (1997). This book describes the loads acting on implants as calculated by analytical methods but is rather outdated by more recent results based on *in vivo* measurement.

A summary of the ASTM standards focused on specific topics can be found in ASTM's selected technical papers (STPs). For example, the standards focusing on fatigue and fracture of medical devices are discussed in STP1481 (Mitchell, 2007).

One of the most helpful and exciting books about implant design, materials, and testing is Steven Kurtz's 'UHMWPE Biomaterials Handbook' (Kurtz, 2009). This book covers a large range of implant types and provides a great overview of the recent literature. The contents of this book go far beyond the scope implied by the title.

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Biological evaluation and regulation of medical devices in the European Union

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Abstract: This chapter considers biocompatibility as part of the requirements to comply with the EU Medical Device Directives. It gives an EU perspective as seen from an EU conformity assessment body ('notified body'). The regulatory framework within which biocompatibility assessment falls is described, and how regulatory requirements can be met. Some of the pitfalls manufacturers may find in the process are considered, and finally it provides a model for presenting biocompatibility data in a manner which should result in a successful assessment by a notified body.

Key words: Medical Device Directive, 93/42/EEC, conformity assessment, notified body, biocompatibility.

16.1 Introduction

This chapter approaches biocompatibility as part of the requirements to comply with the EU Medical Device Directives (MDDs). Its goal is not to revisit the well articulated use of ISO 10993 presented by other authors in this text, but rather to give an EU perspective as seen from an EU conformity assessment body ('notified body'). It describes the regulatory framework within which biocompatibility assessment falls, how regulatory requirements can be met, and some of the pitfalls manufacturers fall into. Finally it presents a model for presenting biocompatibility data in a manner that should result in a successful assessment by a notified body.

16.2 The regulatory and legislative framework

Prior to 1 January 1993, each member state in the EU controlled the safety and marketing of medical devices in its territory in different ways. The introduction of a number of directives (covering such diverse products as toys, recreational craft and civil explosives, as well as medical devices), removed the barriers presented by divergent national technical standards and regulations, and presented a regulatory framework that allowed manufacturers (both within the EU and beyond) to use a single system to bring their

products to market. This system is the ‘new approach directives’, whose fundamental goal is to allow free movement of goods within the single market. The mechanisms used to achieve that goal are based on prevention of new barriers to trade, mutual recognition and technical harmonisation. (Guide to the implementation of directives based on the new approach and the global approach, 2000.)

The medical device regulations are part of this group of new approach directives, and cover all medical devices, divided into three directives. These directives are specific to particular device types as defined within the scope of each directive.

16.2.1 Active implantable medical devices

‘Active implantable medical device’ means any active medical device which is intended to be totally or partially introduced, surgically or medically, into the human body or by medical intervention into a natural orifice, and which is intended to remain after the procedure.

These products are governed by the active implantable medical devices directive (AIMDD) 90/385/EEC, which covers all powered implants or partial implants that are left in the human body (e.g. heart pacemakers).

16.2.2 Medical devices

‘Medical device’ means any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease;
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap;
- investigation, replacement or modification of the anatomy or of a physiological process;
- control of conception.

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

These products are governed by the MDD 93/42/EEC, which encompasses the majority of devices, ranging from ‘simple’ products such as tongue depressors and support bandages, to complex orthopaedic implants, CAT scanners and heart valves. It excludes those products that fall within the scope of either 90/385/EEC or 98/79/EC.

16.2.3 *In vitro* diagnostic medical devices

‘*In vitro* diagnostic medical device’ means any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, equipment or system, whether used alone or in combination, intended by the manufacturer to be used *in vitro* for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for purpose of providing information:

- concerning a physiological or pathological state, or
 - concerning a congenital abnormality, or
 - to determine the safety and compatibility with potential recipients,
- or
- to monitor therapeutic measures.

These products are governed by the *In Vitro* Diagnostic Medical Devices Directive (IVDD) 98/79/EC, which addresses medical devices used away from the patient to make a diagnosis of patient medical conditions, such as HIV test kits and pregnancy test kits.

All of these directives are CE marking directives, i.e. the manufacturer applies the ‘CE mark’ to indicate the relevant essential requirements (ERs) are met and the product is fit for its intended purpose as specified by the manufacturer.

This chapter focuses on requirements for biocompatibility as part of the process of meeting the requirements of the MDD, although the principles applied are similarly applicable to the AIMDD. As biocompatibility is only relevant for medical devices in direct or indirect patient contact, products which fall within the scope of the IVDD directive are not addressed.

The MDDs have a number of standard elements, but there are two specific areas relevant to how biocompatibility assessments support the process of CE marking medical devices. These are the essential requirements, and the presumption of conformity route to demonstrating compliance with the requirements of the directive.

16.3 Essential requirements

Each directive lays down a set of ‘essential requirements’ that have to be met to allow CE marking.

These provide and ensure a high level of protection, and must be applied as a function of the hazard inherent to a given product. Essential requirements define the results to be attained (proportional to the inherent hazards

of the product), but do not specify or predict the technical solutions for doing so. This flexibility allows the manufacturer to choose the way to meet the requirements; it enables the system to be adaptable to innovation and technical progress, but it can be frustrating for device manufacturers who may say 'just tell me what I need to do to comply'.

The essential requirements articulate the fundamental approach to the whole of the product life cycle, with risk management at its heart, and identify all that is necessary to achieve the objective of the directive. Products may be placed on the market and put into service *only* if they are in compliance with the essential requirements.

The essential requirements most pertinent to biocompatibility from 93/42/EEC are abstracted below, but for a more complete understanding of the process the reader should refer to the complete directive.

16.3.1 Essential requirements related to biocompatibility from the Medical Device Directive 93/42/EEC

I. GENERAL REQUIREMENTS

1. The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons, provided that any risks which may be associated with their intended use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety.

This shall include:

- reducing, as far as possible, the risk of use error due to the ergonomic features of the device and the environment in which the device is intended to be used (design for patient safety), and
 - consideration of the technical knowledge, experience, education and training and where applicable the medical and physical conditions of intended users (design for lay, professional, disabled or other users).
2. The solutions adopted by the manufacturer for the design and construction of the devices must conform to safety principles, taking account of the generally acknowledged state of the art.

In selecting the most appropriate solutions, the manufacturer must apply the following principles in the following order:

- eliminate or reduce risks as far as possible (inherently safe design and construction),
 - where appropriate take adequate protection measures including alarms if necessary, in relation to risks that cannot be eliminated,
 - inform users of the residual risks due to any shortcomings of the protection measures adopted.
6. Any undesirable side-effect must constitute an acceptable risk when weighed against the performances intended.

II. REQUIREMENTS REGARDING DESIGN AND CONSTRUCTION

7. Chemical, physical and biological properties

- 7.1 The devices must be designed and manufactured in such a way as to guarantee the characteristics and performances referred to in Section I on the 'General requirements'. Particular attention must be paid to:
- the choice of materials used, particularly as regards toxicity and, where appropriate, flammability,
 - the compatibility between the materials used and biological tissues, cells and body fluids, taking account of the intended purpose of the device,
 - where appropriate, the results of biophysical or modelling research whose validity has been demonstrated beforehand.
- 7.2 The devices must be designed, manufactured and packed in such a way as to minimize the risk posed by contaminants and residues to the persons involved in the transport, storage and use of the devices and to the patients, taking account of the intended purpose of the product. Particular attention must be paid to the tissues exposed and to the duration and frequency of exposure.
- 7.5 The devices must be designed and manufactured in such a way as to reduce to a minimum the risks posed by substances leaking from the device. Special attention shall be given to substances which are carcinogenic, mutagenic or toxic to reproduction, in accordance with Annex I to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Risk management is fundamental to meeting these essential requirements, and it is noteworthy that the hierarchy of risk control articulated in the

directive is the same as that detailed with the harmonised standard EN ISO 14971:2009.

16.4 Presumption of conformity

As the new approach directives do not specify the technical solutions to be achieved, but rather give a framework of necessary elements required, the directives allow manufacturers to demonstrate they meet the essential requirements by the route of ‘presumption of conformity’.

Article 5 – Reference to standards of 93/42/EEC states

1. Member States shall presume compliance with the essential requirements referred to in Article 3 in respect of devices which are in conformity with the relevant national standards adopted pursuant to the harmonized standards the references of which have been published in the Official Journal of the European Communities; Member States shall publish the references of such national standards.

Harmonised standards are European standards, which are adopted by European standards organisations, prepared in accordance with the General Guidelines agreed between the European Commission and the European standards organisations, and follow a mandate issued by the European Commission after consultation with Member States. Harmonised standards are published in the official Journal, and listed on the European Commission website (the harmonised standards applicable to 93/42/EEC are listed at <http://ec.europa.eu/enterprise/policies/european-standards/documents/harmonised-standards-legislation/list-references/medical-devices/>).

Thus the broad requirements articulated in the essential requirements of the directive can be practically addressed by using harmonised standards.

It is important to note that application of harmonised standards, which give a presumption of conformity, is voluntary, and the legal manufacturer can choose an alternative route to demonstrate the product meets the essential requirements. Furthermore, if harmonised standards are used, if appropriate (and suitably justified) the manufacturer can choose to only apply partially apply the standard.

However, in nearly all cases, the application of harmonised standards can help the legal manufacturer to meet the essential requirements, and even in the cases where there may not be product or vertical standards, the existing standards can be used to roadmap requirements. Thus the generic biocompatibility requirements for medical devices as laid out in the essential requirements can be demonstrated specifically by using an appropriate

harmonised standard. In this way EN ISO 10993 can be used to meet the essential requirements.

16.5 Using the EN ISO 10993 series of standards to meet the essential requirements

At the time of writing, 15 of the 20 published parts of the ISO 10993 series of standards are harmonised, including the most important strategic document EN ISO 10993, part 1. Annex ZA from EN ISO 10993-1:2009 details the relationship between the standard and MDD 93/42/EEC, and similarly Annex ZB shows the relationship with AIMDD 90/385/EEC. The tabulation in Annex ZA presents the relevant clause(s)/subclause(s) of the International Standard, and the corresponding essential requirements as summarised in Table 16.1.

Thus Annex ZA indicates that if the biological evaluation is performed according to the clauses listed, the legal manufacturer can state a ‘presumption of conformity’, and use the compliance with ISO 10993 as a route to demonstrate Essential Requirements 7.1, 7.2 and 7.5 have been achieved. The Annex ZA does also carry the caveat that ‘other requirements and other EU Directives may be applicable to the product(s) falling within the scope of this International Standard’, indicating the manufacturer should consider their device within the complete regulatory framework, and ensure all requirements are addressed.

The other parts of ISO 10993 that have been harmonised similarly detail the relationship between the essential requirements and the standard, but generally have a qualifying remark indicating the requirements can only be partly addressed by the International Standard, and that presumption of conformity depends on also complying with all the relevant clauses/sub-clauses of ISO 10993-1. Thus the manufacturer is guided to EN ISO 10993-1, to ensure a complete evaluation is performed.

Table 16.1 Using the EN ISO 10993 series of standards to meet the essential requirements

| Clause(s)/subclause(s) of EN ISO 10993-1:2009 | Essential requirements of Directive 93/42/EEC on medical devices |
|--|--|
| Clause 4: General principles applying to biological evaluation of medical devices Clause 5: Categorization of medical devices Clause 6: Biological evaluation process Clause 7: Interpretation of biological evaluation data and overall biological safety assessment | Annex I:7.1, 7.2 and 7.5 |

16.5.1 Practical application of harmonised standards to demonstrate compliance with essential requirements

The previous sections have described the EU regulatory landscape within which biocompatibility assessment operates, and the regulatory compliance rationale for ensuring products are appropriately biocompatible. How then does the implicit requirement to ensure a medical device is biocompatible translate into the practical approach the legal manufacturer undertakes in developing a medical device?

When the 4th edition EN ISO 10993-1:2009 superseded the 3rd edition of the standard (EN ISO 10993-1:2003), the new version much more clearly emphasised the biological evaluation as part of the overall risk management process. Thus prior to looking in detail at EN ISO 10993, the manufacturer must turn to the fundamental horizontal standard EN ISO 14971:2009. Horizontal standards (also known as basic standards) cover common requirements for all or a wide range of medical device areas such as risk management, labelling and sterilisation methods. Since risk management is fundamental to the essential requirements, this standard lays out requirements for the risk management process for all medical devices. It articulates the need to consider all hazards appropriate for a medical device, and provides specific details addressing the risk analysis process for biological hazards (Annex I of the standard). Annex I presents a very short guidance, which gives some of the basic principles, but in essence points the manufacturer to the ISO 10993 series of standards.

A complete risk management report (the output from the risk management process) will always form part of the technical documentation which demonstrates the essential requirements of MDD 93/42/EEC have been met. This document should present a comprehensive assessment of the hazards identified, the risk control measures taken, and overall acceptability of residual risk. However this risk management report is the final output from the risk management process that should operate throughout the development of the product (and the risk management process will continue for the whole of the product life cycle).

Thus biological hazards should be clearly identified within the risk management report, with an appropriate risk evaluation and detailed risk control measures. This process has been covered in detail in other chapters, and the reader is directed to Annex B of EN ISO 10993-1:2009 for guidance on how the biological evaluation fits within the risk management framework.

16.5.2 Conformity assessment process

Annex IX of MDD 93/42/EEC sets out the classification rules which manufacturers should use to determine which class a device falls into, according

to its properties, function and intended purpose. Devices are assigned to one of four classes: Class I for low-risk devices, Classes IIa and IIb for medium-risk devices and Class III for high-risk devices. It is important to note that the device classification required by 93/42/EEC is not the same as that utilised within EN ISO 10993-1:2009, and the two classification processes are entirely independent of each other.

As previously stated all devices must meet the essential requirements of the MDD, irrespective of device class, however there are different conformity assessment routes to demonstrate compliance. In the case of Class I devices that are non-measuring and non-sterile the manufacturer is responsible for ensuring his product complies with the relevant essential requirements of the directive, and makes a self declaration to that effect.

All other devices (i.e. Class I measuring or sterile devices, Class IIa, IIb and III) require conformity assessment by a notified body. The type and depth of assessment varies dependent on the classification of the device, but in all cases the notified body will review the technical file/design dossier for a medical device, and judge whether the product meets the essential requirements. Thus the biocompatibility assessment for the medical device will be reviewed by a notified body.

16.6 The notified body

A notified body is a certification organisation that the national authority (the Competent Authority) of an EU Member State designates to carry out one or more of the conformity assessment procedures described in the annexes of the MDD 93/42/EEC. A notified body will be qualified to perform all the functions set out in any annex for which it is designated, and the designation may be restricted to specified types of devices and/or Annexes.

At the time of writing there are 76 notified bodies designated to perform conformity assessments according to 93/42/EEC within the EU. Notified bodies can have varying levels of experience and expertise, so it is important that the notified body working with the manufacturer has the appropriate knowledge to assess the biocompatibility of the type of device in question. There are many factors to consider when selecting a notified body, which are outside the scope of discussion related specifically to biocompatibility, so Table 16.2 illustrates some key points to consider as part of the notified body selection process.

If the manufacturer has chosen to utilise the presumption of conformity route by using EN ISO 10993 to demonstrate compliance with the relevant essential requirements, the task of the notified body is relatively simple, which is to determine whether the manufacturer has complied with the requirements of the harmonised standard. A later section deals with

Table 16.2 Key points to consider as part of the notified body (NB) selection process

| Resource | Working together | Technical | Commercial |
|---|---|--|---|
| <ul style="list-style-type: none">• Standing and reputation | <ul style="list-style-type: none">• Effective working relationship | <ul style="list-style-type: none">• Expertise in product area | <ul style="list-style-type: none">• Costs |
| <ul style="list-style-type: none">• Designating competent authority | <ul style="list-style-type: none">• Flexibility/responsiveness | <ul style="list-style-type: none">• Expertise in biocompatibility | <ul style="list-style-type: none">• Timelines |
| <ul style="list-style-type: none">• Scope of certification schemes offered (global access to markets) | <ul style="list-style-type: none">• Willingness to support the manufacturer | <ul style="list-style-type: none">• Format/content of NB audit reports | <ul style="list-style-type: none">• Ongoing costs |
| <ul style="list-style-type: none">• Size | <ul style="list-style-type: none">• Willingness to meet | <ul style="list-style-type: none">• Clinical evaluation expectations | |
| <ul style="list-style-type: none">• Location | <ul style="list-style-type: none">• Accessibility of personnel | <ul style="list-style-type: none">• Documentation requirements | |
| <ul style="list-style-type: none">• Use of subcontractors | <ul style="list-style-type: none">• Approachability | | |
| <ul style="list-style-type: none">• Audit schedule | <ul style="list-style-type: none">• Pre-audit review | | |

some of the common pitfalls manufacturers can fall into in submissions, and provides an example of the appropriate content of a biocompatibility assessment.

In the rare cases where a manufacturer has not chosen to use the presumption of conformity route to demonstrate the product meets the essential requirements related to biocompatibility, it is likely that the notified body will continue to use the harmonised standards as a framework for the assessment, although they cannot audit precisely against the requirements of the relevant harmonised standard. This can lead to a more protracted review process (with potential time and cost implications).

Whilst the presumption of conformity route indicates that compliance with the appropriate harmonised standard is satisfactory to meet the essential requirements identified, it cannot be overstated how important it is to utilise the harmonised standard robustly. This is particularly relevant where no vertical (product) standard exists within the EN ISO 10993 series. The risk analysis of the medical device may identify hazards that should be addressed in addition to those described within EN ISO 10993-1 2009. This issue is addressed in more detail later in the chapter.

16.6.1 Technical file evaluation by the notified body

The technical file (or design dossier for Class III devices) holds all the relevant information that demonstrates conformity of the medical device to the

Table 16.3 An example format for addressing the relevant essential requirements (ER) from Annex I of the directive

| Essential requirement | Applicable to the device | Harmonised standard(s) applied | Standards applied partially or in full | Compliance demonstrated by/comments | Location in technical documentation |
|-------------------------------------|--------------------------|---|--|--|--|
| 7.1 (text of ER can be included) | Yes | EN ISO 14971:2009 EN ISO 10993-1:2009 EN ISO 10993-5:2009 EN ISO 10993-18:2009 | Fully applied | - Risk management report - Biocompatibility assessment - Cytotoxicity report | Section X Risk Management Section Y Biocompatibility assessment Appendix Z Test reports |

requirements of the directive (i.e. that all relevant essential requirements are met). The directive does not give specific guidance on the content of the technical file, so the manufacturer can construct the file as appropriate. However, manufacturers may chose to use a standardised format such as the Summary Technical Documentation as described by Global Harmonisation Task Force (GHTF) document SG1 (PD)/N011R20: STED), which will be helpful for the notified body assessment process.

For the purposes of the conformity assessment against the essential requirements of 93/42/EEC related to biocompatibility, there are three documents which the reviewer will focus on.

The essential requirements checklist

This roadmaps how the manufacturer has addressed the relevant essential requirements from Annex I of the directive. Table 16.3 presents an example format.

In this case the manufacturer has clearly indicated the presumption of conformity route has been used to demonstrate how the essential requirement is met, thus the notified body reviewer can easily identify the relevant information to review.

The risk management report

As biocompatibility forms part of the overarching risk management process the risk management report should clearly identify the biological safety hazards and the risk control measures taken to address the risks identified.

The biocompatibility assessment

The documentation that addresses the biological evaluation according to EN ISO 10993

When the manufacturer is preparing the biological evaluation, the regulatory authority/notified body assessor is one of the important stakeholders, so it is helpful to consider their needs. With all regulatory submissions (whether in the EU or worldwide), the regulatory authority is a key customer, and it remains surprising how many manufacturers do not consider the needs of the reader when preparing their technical documentation. Ideally the notified body will have appropriate expertise specific to your type of device, but that is not always the case. Depending on the product type and classification the whole technical file may be viewed by one expert, but in some cases the relevant sections of the technical file will be assessed by an expert in biocompatibility, without sight of the whole technical file. Thus the biocompatibility assessment may need to be auditable in isolation from other technical documentation, such as design verification and validation activities.

The points to consider when preparing biological safety documentation for review are listed below:

- the assessor may or may not be an expert in biocompatibility;
- they will know the technology area;
- they may not know your product in detail;
- they may not know the history of your product;
- they will probably be reviewing against the requirements of the EN ISO 10993 series of standards;
- they may be considering biocompatibility as part of overall submission;
- they will be looking for a coherent thread between risk management and the biological evaluation;
- they will be skilled in identifying data gaps, inconsistencies and insufficient/poor quality data.

Use of non-validated/non-standard test methodologies

Various parts of the ISO 10993 series of standards describe in some detail validated test methodologies, and these should always be the preferred route for demonstrating compliance with the standard. However, it is not so prescriptive as to limit the biological evaluation to only those test methodologies described within the standard, but it is clear that all testing should be scientifically robust. Thus there may be instances where ‘non-standard’ test methodologies are required, and this is acceptable if duly justified (particularly when novel/innovative products are under assessment). However, it is not appropriate to present a biological evaluation using a non-validated

methodology (such as a novel cytotoxicity test developed in a university laboratory), when the evaluation could be performed using a recognised validated methodology as described in the EN ISO 10993-5). That does not preclude the use of the data generated in the university methodology as part of the background to the biological evaluation, but it should not be used as the pivotal data set.

Use of combined performance and safety tests

Whilst the primary aim of the ISO 10993 standards is the protection of humans, it also serves as a framework to minimise the number and exposure of test animals, thus where appropriate the combining of studies to address multiple endpoints is encouraged. However this is subject to the primary endpoints of those tests not being compromised by the secondary endpoint. An example of such a study would be a performance evaluation of an orthopaedic implant in a large animal model. Whilst the primary goal of the study might be to assess mechanical performance of the implant, analysis of serum ion levels could provide supporting evidence for the biocompatibility of the medical device.

Similarly for drug device combination products (e.g. drug eluting stents), such combined safety and performance tests can provide valuable information on both the performance of the device component, and also relevant pharmacokinetic data to support drug safety and efficacy parameters.

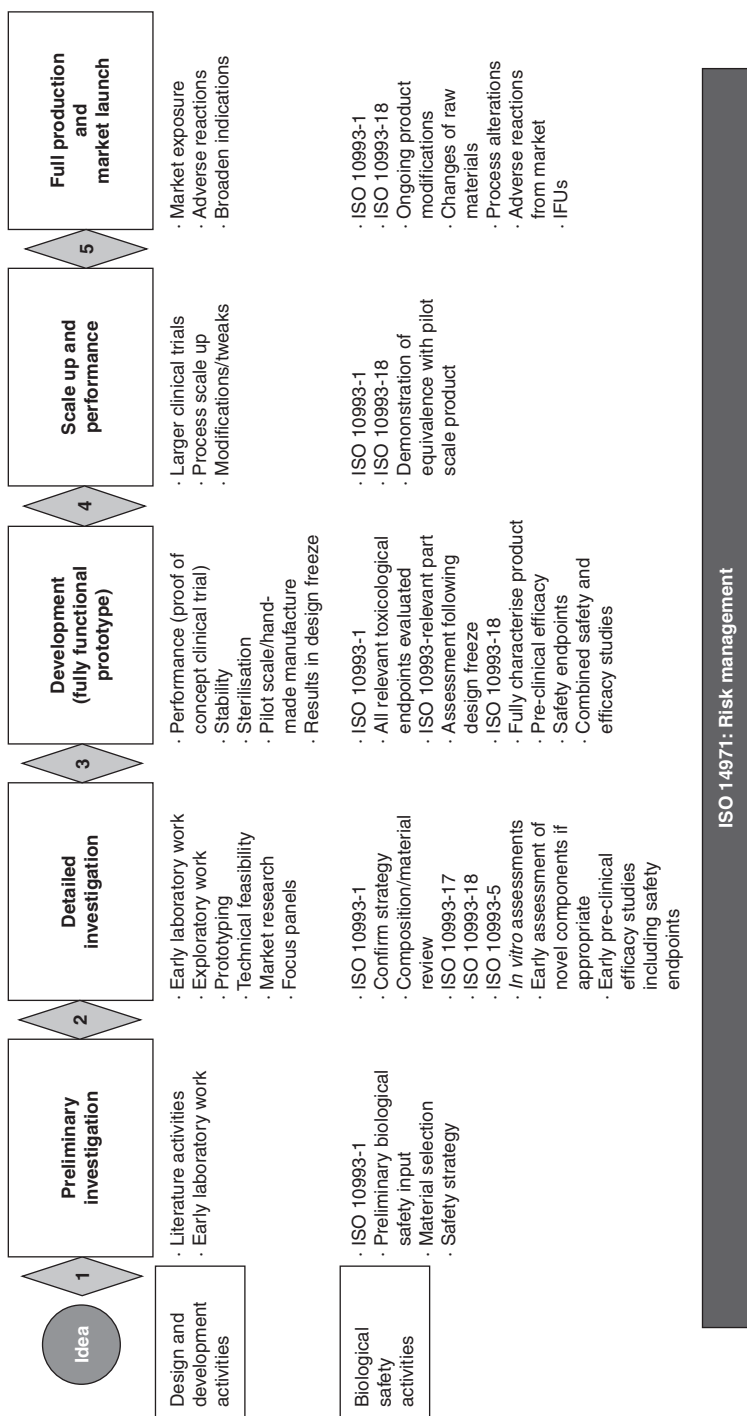
16.7 Common pitfalls in biological evaluations

The most fundamental problem observed in some manufacturer's submissions is the lack of a coherent risk management process, and the biocompatibility assessment, which should be aligned to it. In these cases it appears the risk management process is not at the heart of the device development, but rather a 'bolt on' performed to demonstrate regulatory compliance. Thus the risk analysis does not identify all the relevant hazards, and the risk control measures, whilst being valid activities as part of design and development, do not demonstrate management of the risks identified.

In these cases the biological hazards are not clearly identified, and there appears no rationale for the subsequent biological evaluation performed.

Figure 16.1 presents an example of the product-development process, with risk management operating as the foundation for the product-development process. Over-layered on the risk management are the interactions with the biocompatibility assessment and the new product-development process.

To highlight the interrelationship between risk management and biological safety Table 16.4 presents an example of the hazards identified for a wound dressing to be applied to chronic wounds, identified as part of the EN ISO 14971:2009 risk management process.



16.1 An example of some of the relationships between the new-product-development process and biological evaluation. At various stages within the development process there are key inputs where the biological evaluation is critical to the successful development of the product. Underlying all activities, risk management forms the foundation for an effective and efficient new-product-development process (IFUs, instruction for use).

Table 16.4 An example of the hazards identified for a wound dressing to be applied to chronic wounds, identified as part of the EN ISO 14971:2009 risk management process

| Hazard | Foreseeable sequence of events | Hazardous situation | Harm |
|---------------------------------|---|--|---|
| Chemical: Device composition | Polyurethane materials present in device do not have appropriate biocompatibility | Leachable polymeric components have an adverse effect either locally or systemically | <ul style="list-style-type: none"> • Pain • Local irritation • Inflammatory response • Sensitisation • Delayed wound healing • Target organ toxicity • Genetic damage leading to carcinogenicity |
| | Physico-chemical characteristics of materials not appropriate for contact with wound tissue | Wound contact materials adhere to wound bed, causing damage on removal | <ul style="list-style-type: none"> • Pain • Local irritation • Inflammatory response • Delayed wound healing |

The hazards described fall under the broad description of ‘biocompatible’. However, a common misconception of manufacturers is that a series of biocompatibility tests, as described by EN ISO 10993-1:2009, demonstrates that a product is suitably biocompatible.

Using the guidance presented in Annex A of EN ISO 10993-1:2009, the following evaluation tests would be considered appropriate for breached or compromised surface contact device (contact duration C – permanent), and would form part of the hazard characterisation and risk control activities:

- cytotoxicity (EN ISO 10993-5),
- sensitisation (EN ISO 10993-10),
- irritation or intracutaneous reactivity (EN ISO 10993-10),
- subchronic toxicity (subacute toxicity) (EN ISO 10993-11),
- genotoxicity (EN ISO 10993-3).

Whilst these assessments would characterise the risks for those toxicological endpoints, they do not completely address the hazardous situations identified, as delayed wound healing cannot be assessed by the evaluations described. Therefore the manufacturer may need to consider an additional evaluation

not described within the ISO 10993 series of standards, and in this case a wound healing evaluation in an appropriate model would be required to adequately address the risks identified within the risk management process.

This point underlines the need for thorough hazard identification within the risk management process (in this case the fundamental performance requirement of a chronic wound dressing that it does not cause a delay in wound healing or significant damage to the wound bed upon removal was ignored). Once appropriate risks are identified, they may well be addressed by using harmonised standards, but those standards should not be utilised in a 'blinkered' manner. It is clear from EN ISO 10993-1 that the standard provides a framework for the biological evaluation, and not a rigid test regime, and it is specifically stated within the standard that it is not intended to provide a rigid set of tests methods, including pass/fail criteria.

Within the biological evaluation itself, the most common issue is inadequate/incomplete application of EN ISO 10993-1 2009. The standard presents a process flow for a systematic approach to the biological evaluation, which if followed provides a complete evaluation. Unfortunately some manufacturers may view a biocompatibility assessment purely as 'the tests', and whilst this view may not be promulgated by contract research laboratories performing testing, it can be perpetuated by the lack of a complete systematic approach encompassing all aspects of the biological evaluation. This can be particularly prevalent in small and medium enterprises (SMEs), where dedicated expertise in biological safety is not available.

Whilst manufacturers may not have the expertise in house to complete the biological evaluation, as the legal manufacturer of the medical device under the requirements of the MDD 93/42/EEC it is their responsibility to ensure the product has a complete biological evaluation and thus meets the essential requirements. The ownership of the biological evaluation process remains with the manufacturer (even if the whole biological evaluation is subcontracted to a contract house or a medical device consultant). Thus the fact the supplier of a component material states it is 'biocompatible', and may present test data, does not abrogate the responsibility of the legal manufacturer for the biological safety of the finished product. In this case the validity of that data for the finished product use will be challenged, which brings the challenge for the manufacturer of justifying toxicological equivalence.

The 4th edition of EN ISO 10993-1:2009 brought welcome clarification to manufacturers, clearly allowing the use of existing preclinical and clinical data, including history of safe use, to meet the requirements of biological evaluation. Thus the use of existing data is encouraged, to avoid unethical additional animal testing (which also has the benefits of reduced time and cost to manufacturers), however the challenge of justifying the acceptability of existing data for a new medical device can be significant. The use of chemical characterisation (EN ISO 10993-18) facilitates demonstration

of chemical equivalence, which can then be extrapolated to toxicological equivalence (refer to Annex C of part 18 for further detail). Common examples where such toxicological equivalence can be used are generic components that can be used by many different manufacturers (e.g. adhesives for wound dressings, or polyether ether ketone (PEEK) polymer for implantable devices). In these cases the supplier may state the materials are 'biocompatible', and have specific test data relevant to the manufacturer's end use. However, the processing, sterilisation and finished device use are controlled by the manufacturer, which will directly impact on the overall biocompatibility of the medical device. In these instances the manufacturer must justify why the data presented by the supplier (e.g. a polymer sterilised by ethylene oxide), is acceptable for the end use (where the manufacturer may have chosen to use an alternative method of sterilisation such as gamma irradiation). Particularly in the case of polymeric materials, where molecular weight/monomer content can have a significant impact on the biocompatibility of the material, it is likely that chemical characterisation will form part of the justification for equivalence.

Further examples of inadequate biological evaluation submissions include those with simple unsupported statements such as 'it's only material x, it's well known to be safe', 'my material is approved by y', 'my material complies with an ISO standard', 'this material has been used safely for years'. Whilst all of these top level statements may be relevant to the overall biological evaluation, they do not stand as an acceptable evaluation alone (and must be supported by objective evidence, not anecdotal comment). EN ISO 10993-1:2009 encourages the use of existing data, but only within the overall framework of the biological evaluation. Similarly, the observation 'my competitor's product contains the same material so it must be biocompatible' is not an acceptable justification of equivalence without substantial supporting evidence and utilisation of biocompatibility data from another source. All of these shortfalls result from inadequate application of the harmonised standard.

16.8 Managing positive results in the biological safety assessment

EN ISO 10993-1:2009 emphasises the biological evaluation within the risk management process, and highlights that the standard presents a framework for the biological evaluation, without pass/fail criteria. Similarly as part of the overall risk management process for a medical device, it is the manufacturer's responsibility to set acceptable risk levels based on state of the art. Thus, if appropriate, positive or equivocal results from biocompatibility studies may be deemed acceptable within the overall biological evaluation. In these instances it is important the manufacturer clearly presents the

data, with a rationale or hypothesis for the results obtained, and if possible further analysis (perhaps chemical characterisation) to suggest why such a result has been achieved. Two examples are presented below, but there may often be situations where one test performed presents a problematic result. In these situations it is critical the manufacturer presents a robust justification for the acceptability within the overall biological evaluation, and does not ignore the data.

Positive test results can often occur when sensitive *in vitro* evaluations are performed due to the absence of normal physiological and morphological protective mechanisms (e.g. cytotoxicity in a EN ISO 10993-5:2009 MEM elution assay when evaluating a silver wound dressing), which although providing some hazard identification do not preclude the use of the product if other more relevant *in vivo* data exists. In the example of the silver dressing the cytotoxicity demonstrated is an inevitable consequence of the silver antimicrobial, but the *in vitro* cytotoxicity does not translate into adverse wound effects in either preclinical porcine models or clinical use. The acceptability of a positive result must be presented as part of the overall risk management assessment, and in that context the biocompatibility of the device may still be acceptable for the clinical use intended.

The use of aggressive solvent extraction methodologies as recommended in the Japanese *in vitro* cytotoxicity and genotoxicity assays, and the guinea pig sensitisation test, can lead to positive results in these tests, which may not be observed when the physiological solvents described in EN ISO 10993-12 2009 are used. Whilst in some instances the results may be a true representation of the biological hazard presented, in other cases the aggressive solvent extraction can lead to the presence of various moieties that would not be present in normal clinical use, and can be considered 'false positives'. In this situation it may be appropriate to justify the acceptability of these results when presented in the context of additional testing with physiological media and chemical characterisation demonstrating the discrepancies between aggressive solvent extracts and physiological extracts.

16.9 Presenting the biological evaluation within the technical file

As previously discussed, for any regulatory submission it is important to consider the key stakeholders of the information presented. In the case of the biological evaluation the assessor will invariably be assessing the content against EN ISO 10993-1 2009, thus presenting the information in a format consistent with the process flow of the standard will facilitate the process. The Appendix in Section 16.12 illustrates a model presentation of data, and reiterates that the biological evaluation is not just 'the tests', but a structured evaluation of the data relevant to biological safety.

16.10 Conclusion

Although the MDD requirements can appear convoluted and confusing, there are clear mechanisms to demonstrate the acceptable biocompatibility of medical devices. The use of the ‘presumption of conformity’ process allows the use of international standards that have been harmonised within the EU, thus enabling manufacturers within the EU and beyond to have a consistent approach to biocompatibility.

Robust utilisation of both the risk management standard EN ISO 14971:2009 and EN ISO 10993 series of standards will ensure a thorough biocompatibility assessment is performed, which should lead to a successful assessment of the submission by the notified body.

16.11 Sources of further information and advice

- European Commission Guide to the Implementation of Directives Based on New Approach and Global Approach
<http://ec.europa.eu/enterprise/policies/single-market-goods/documents/blue-guide/>
- European Commission Website for Medical Devices, providing extensive guidance documents, including MEDDEVs, borderline considerations and consensus statements
http://ec.europa.eu/health/medical-devices/index_en.htm
- European Commission Website of Harmonised Standards for Medical Devices
http://ec.europa.eu/enterprise/policies/european-standards/harmonised-standards/medical-devices/index_en.htm
- European Commission Website “NANDO”, detailing designation of notified bodies for MDDs
<http://ec.europa.eu/enterprise/newapproach/nando/>
- Notified Body Operations Group Website, detailing various recommendations for processes and documentation
<http://www.nbog.eu/>

16.12 Appendix: model content of the biological evaluation submission

- Product construction/composition
 - Intended use
 - List components/materials with direct/indirect body contact
 - Diagram of product
 - Auxiliary materials – additives, processing aids, residues
 - Sterilisation methodology

- EN ISO 10993:1 Classification of device
 - Nature of contact
 - Duration of contact
- Product characterisation
 - ISO 10993-18/19
 - If appropriate analysis of degradation products (EN ISO 10993-9)
 - If appropriate establishment of allowable limits of leachable substances ((EN ISO 10993-17)
- Gap analysis – similarity to other devices (if relevant)
 - Intended use /device properties
 - Components
 - Manufacturing
 - Sterilisation methodology
- Historical data review
 - Literature (from legitimate sources such as the Hazardous Substances Database, Toxline, National Toxicology Program etc. Wikipedia is not considered a robust source)
 - Relevance of literature
 - Body contact
 - Duration
- Pre-existing internal company information
 - Robust database
 - Supporting evidence
 - Preclinical tests from similar devices
 - Relevance to the device under assessment
- Selection of biological evaluation test strategy
 - Rationale
 - Justification for tests performed/not performed
 - Description of tested items
 - Representative sample
 - Finished device, part of device, raw material
 - Sample preparation, solvent
 - Sterilisation of material
- Preclinical test results
 - Summary tabulation
 - Full test reports supplied in appendices

| Test sample | Status of test material | Sample/ extract preparation | Test performed/ test system | Standard/ norm | Test lab/report number/report date | Test result |
|-------------|-------------------------|-----------------------------|-----------------------------|----------------|------------------------------------|-------------|
|-------------|-------------------------|-----------------------------|-----------------------------|----------------|------------------------------------|-------------|

- Conclusion
 - Summary of biological evaluation strategy and results
 - Clear discussion of all data including equivocal results, justify their acceptability
 - Statement of meeting requirements of EN ISO 10993
 - Evidence of incorporation into risk management documentation

Biological evaluation and regulation of medical devices in Japan

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Abstract: In Japan, unified guidelines for biological safety tests of medical devices have been implemented since 1995. Since then, there have been no marked changes in the basic way of thinking. In recent years, Japan's way of thinking has been introduced into various parts of the ISO 10993 series, and international understanding has also been promoted. The basic test methods used in Japan do not differ from those used outside Japan for most test items. However, there is a perception gap in sample preparation applied to these tests. In Japan, the basic stance is to eliminate all risks systematically. This stance is especially prominent in cytotoxicity tests, sensitization tests and genotoxicity tests. In addition, there is a perception gap also in classification. Focusing on these points, this chapter outlines the Japanese guidance for biological safety tests of medical devices.

Key words: colony formation, cytotoxicity, skin sensitization, genotoxicity, classification, exaggerated extraction, extracts from polymeric materials.

17.1 Introduction

In 1995, the Ministry of Health and Welfare issued Yakuki Notification No.99 'Guidelines for Biological Safety Tests Required When Applying for Approval to Manufacture (Import) Medical Devices',¹ Japan's first uniform guideline for implementing tests on the biological safety of medical devices, tests that were previously conducted under individual regulations. As the first version of ISO 10993-1 had already gone into effect in 1992, there was a three-year gap between the two guidelines. 'Basic Considerations on Biological Safety Evaluation of Medical Devices'² (hereinafter referred to as 'Iyakushin Notification No.0213001'), an appendix to 'Basic Considerations on Biological Safety Tests Required When Applying for Approval to Manufacture (Import) Medical Devices', was then issued in 2003 as a revision to Yakuki Notification No.99. At almost the same time, 'Test Methodology for the Biological Evaluation of Medical Devices' (hereinafter referred to as 'Iryokiki-Shinsa No.36') was attached and issued as an appendix to 'Reference for Basic Considerations on Biological Safety Tests'.³ Iryokiki-Shinsa No.36 is

the equivalent of a portion of the items for each type of test in the ISO 10993 series. Eight years have passed since the enactment of Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36, and work on revisions is ongoing. Though the final detail of those revisions is not yet clear as of the writing of this paper, one can venture a guess that, at present, there will not be any major changes to Japan's basic position on biological-safety tests.

Meanwhile, standards on the implementation of non-clinical studies of medical devices have been provided in order to enable testing facilities that perform such non-clinical tests to offer reliable tests conducted from the standpoint of both software and hardware. Those standards were the so-called GLP (Good Laboratory Practice) and the 'Standard for the Conduct of Non-clinical Safety Studies of Medical Devices' (Iyaku Notification No.0930001),⁴ which were issued and enacted for medical devices in 2002. Following that was Ministry of Health, Labor and Welfare Ordinance No.37 'Ordinance on the Standard for the Conduct of Non-clinical Safety Studies of Medical Devices',⁵ in 2005.

In this chapter, we examine the manner of conducting biological-safety tests on medical devices and medical materials and how to make the best use of that in applications for approval to manufacture and distribute based upon Japan's Medical Device GLP and centered on the content in Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36. We also discuss the ISO 10993 series international standards on medical devices and the related ASTM series standard in the United States, while touching on their connections and differences.

17.2 Outline of biological safety testing in Japan

Content-wise, Iyakushin Notification No.0213001 is on the same lines as ISO 10993-1 (ref. 6), having gathered and reflected on the results of biological safety evaluations conducted under the guidelines of Yakuki Notification No.99, international movements towards the standardization of test methods, and trends towards animal welfare, etc. Its aim is to 'indicate the basic considerations on risk assessment of adverse biological actions (toxic hazards) and biological safety tests as a part of a medical device's pre-marketing safety evaluation'. Iryokiki-Shinsa No.36 details individual test methodologies, and this is seen as an arrangement of concrete examples of sensitive, simple-to-implement test methods based on the results of Yakuki Notification No.99. If a test methodology presented in Iryokiki-Shinsa No.36 is used as a model, or even if another test methodology is used (as long as the validity can be justified based on the considerations in Iyakushin Notification No.0213001), then that test is assumed to be usable. For example, looking at '3. Adoption of International Standards' in Iyakushin Notification No.0213001, it adequately notes implementation under a test methodology described in the ISO 10993

series. In many parts of the ISO 10993 series, multiple test methods are described in parallel. From among these, those for which validity has been verified and for which usage results have already been accumulated domestically are selected and indicated in Japan. As the criteria for selecting the test methodology described in each part of the ISO 10993 series are not necessarily clearly indicated, appropriate test methodology is required based on the descriptions following '4. Principles of Biological Safety Evaluation' in Iyakushin Notification No.0213001.⁷ Specifically, '6. Test Methods' states, 'When there are multiple test methods for a given evaluation item, selection must be done taking into account the principles, sensitivity, selectivity, quantitative capability, and reproducibility of the test methods as well as application method and limitation of test samples, with respect to the significance of the biological safety evaluation for the medical device.' The thinking in Japan is strongly indicated here.

According to Iyakushin Notification No.0213001, the test items shown in Table 17.1 are, as a general rule, required in the primary evaluation for each medical device and material, depending on the categorization in line with the site at which the evaluated medical device or material contacts the body (Table 17.2) and the duration of that contact (Table 17.3). When evaluation is carried out for a medical device or its component material that does not fall into any of the specified categories, the evaluation items that apply to the closest category should be selected. If multiple categories, because of the duration of contact, apply to the device, the item that corresponds to the category with the longer duration should be evaluated. When there are categories applicable to multiple points of contact, the items corresponding to each category should be evaluated. Multiple evaluation items are also enumerated, respectively for irritation tests and blood compatibility tests. Which item to evaluate and which test methodology to use should be determined in line with the actual conditions of usage for each individual medical device. In the case of medical devices with prolonged contact, consideration is called for in regard to whether there is a need to perform supplementary evaluation through tests on chronic toxicity, carcinogenicity, reproductive/developmental toxicity and biodegradation (Table 17.4).

For testing with test animals, there are related laws and guidelines within Japan, such as the 'Act on Welfare and Management of Animals',⁷ 'Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain',⁸ 'Guidelines for Proper Conduct of Animal Experiments',⁹ and 'Basic Guidelines for Conducting Animal Experiments, etc., in Testing Facilities under the Jurisdiction of the Ministry of Health, Labor and Welfare'.¹⁰ Based on these guidelines, internal regulations are provided at each testing facility so that animal testing is conducted appropriately. Moreover, a system is already in operation domestically in which a third-party institute investigates and evaluates the implementation conditions at each facility.

Table 17.1 Guidelines for primary evaluation

| Category of medical device | Biological test | | | | | | | | | |
|--------------------------------|---------------------------------|----------------------|--------------|---------------|--|-------------------------|-------------------|--------------|--------------|---------------------------------|
| | Nature of contact | Duration of contact* | Cytotoxicity | Sensitization | Irritation/ intra-cutaneous reactivity | Acute systemic toxicity | Subacute toxicity | Genotoxicity | Pyrogenicity | Implantation Hemo-compatibility |
| Surface devices | Skin | A | ○ | ○ | ○ | | | | | |
| | | B | ○ | ○ | ○ | | | | | |
| | | C | ○ | ○ | ○ | | | | | |
| | Mucous membrane | A | ○ | ○ | ○ | | | | | |
| | | B | ○ | ○ | ○ | | | | | |
| | | C | ○ | ○ | ○ | | ○ | | | |
| | Breached or compromised surface | A | ○ | ○ | ○ | | | | | |
| | | B | ○ | ○ | ○ | | | | | |
| | | C | ○ | ○ | ○ | | ○ | | | |
| External communicating devices | Blood path, indirect | A | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | | B | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | | C | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | Tissue, bone, or dentin | A | ○ | ○ | ○ | | | | ○ | |
| | | B | ○ | ○ | | | | | | |
| | | C | ○ | ○ | | | | | ○ | |
| | Circulating blood | A | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | | B | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | | C | ○ | ○ | ○ | ○ | ○ | ○ | | ○ |
| Implanted devices | Tissue or bone | A | ○ | ○ | ○ | | | | | |
| | | B | ○ | ○ | | | ○ | | ○ | |
| | | C | ○ | ○ | | | ○ | | ○ | |
| | Blood | A | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | | B | ○ | ○ | ○ | ○ | | ○ | | ○ |
| | | C | ○ | ○ | ○ | ○ | ○ | ○ | | ○ |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

* A: limited contact (within 24 h), B: prolonged contact (1–30 days), C: permanent contact (more than 30 days).

Table 17.2 Categorization by area of body contact of medical device

| | |
|---------------------------------------|---|
| Non-contact devices | Medical devices that do not contact the patient's body directly or indirectly |
| <i>Surface contact devices</i> | |
| Skin | Medical devices that contact intact skin surfaces only |
| Mucous membranes | Medical devices that contact intact mucous membranes such as oral cavity, esophagus and urethra |
| Breached or compromised surfaces | Medical devices that contact breached or otherwise compromised skin or mucous membranes |
| <i>External communicating devices</i> | |
| Blood path, indirect | Medical devices that contact blood path at one point and serve as a conduit for drug entry into the vascular system |
| Tissue, bone, or dentin | Medical devices that contact tissue, bone, or pulp and dentin system |
| Circulating blood | Medical devices that contact circulating blood |
| <i>Implant devices</i> | |
| Tissue or bone | Medical devices that contact tissue and/or bone |
| Blood | Medical devices that principally contact blood |

Table 17.3 Categorization by duration of contact of medical device

| | |
|-------------------|---|
| Limited contact | Medical devices with a contact duration of less than 24 h |
| Prolonged contact | Medical devices of which single, multiple or longer-term use has a contact duration of not less than 24 h and up to 30 days |
| Permanent contact | Medical devices of which single, multiple or long-term use has a contact duration exceeding 30 days |

Though international standardization is being advanced on the quality and feeding methods for test animals, it is also necessary to comply with ISO 10993-2 (ref. 11) when taking medical-device safety testing into consideration. Iyakushin Notification No.0213001 as well, under '8. Animal Welfare', states, 'The treatment of animals used in animal testing is performed according to the Law for the Humane Treatment and Management of Animals and ISO 10993-2 "Animal Welfare Requirements"'.

17.3 Biological safety tests

Safety has been examined on the assumption that all chemical substances, including pharmaceuticals, contain an element that causes an undesirable

Table 17.4 Guidelines for supplemental evaluation

| Category of medical device | | Biological test | | | |
|--------------------------------|---------------------------------|------------------|-----------------|-------------------------------------|----------------|
| Area of contact | Duration of contact* | Chronic toxicity | Carcinogenicity | Reproductive/developmental toxicity | Biodegradation |
| Surface devices | Skin | A | | | |
| | | B | | | |
| | | C | | | |
| | Mucous membrane | A | | | |
| | | B | | | |
| | | C | | | |
| | Breached or compromised surface | A | | | |
| | | B | | | |
| | | C | | | |
| External communicating devices | Blood path, indirect | A | | | |
| | | B | | | |
| | | C | ○ | | |
| | Tissue, bone, or dentin | A | | | |
| | | B | | | |
| | | C | ○ | | |
| | Circulating blood | A | | | |
| | | B | | | |
| | | C | ○ | | |
| Implanted devices | Tissue or bone | A | | | |
| | | B | | | |
| | | C | ○ | | |
| | Blood | A | | | |
| | | B | | ○ | |
| | | C | ○ | | |

* A: limited contact (within 24 h), B: prolonged contact (1–30 days), C: permanent contact (more than 30 days).

effect. Biological safety tests conducted as non-clinical studies using animals and cells, etc., aim at analyzing the conditions and mechanisms in which those effects are manifested, and thus estimate the onset of toxicity. Such testing considers the state of exposure to humans and, along with the confirmation of hazards, it clarifies the dosage–effect relationship and is conducted under the expectation of producing results that can be used as data that are necessary for performing risk assessment. Therefore, it is normal in these biological safety tests to require a dosage group that includes at least the amount at which the onset of toxicity is clearly evident. However, there are many cases with medical devices or medical material when it is in fact difficult to set an amount that results in the onset of toxicity, and this is thought to be a unique aspect of biological safety testing for medical devices. In such cases, tests are conducted using as high an exposure level as possible, and the aim is to judge safety based on those results.

When a biological safety test is conducted on a medical device, the target test samples can be roughly divided into the finished product, its components, raw materials, and raw chemical substances. One feature of medical devices is that many are manufactured by combining multiple parts, raw materials, and raw chemical substances. The definition of a raw material in Iyakushin Notification No.0213001 indicates that it ‘Refers to a material for medical devices or a material used in the manufacturing processes for medical devices (including test/inspection processes and sterilization processes), such as synthetic or natural polymer compounds, metals, alloys, ceramics, and other chemical substances.’ The definition of finished product ‘Refers to a post-test/inspection medical device ready for shipment and, in the case of a sterile product, refers to a product after sterilization.’ Appropriate test samples include finished products, parts of a finished product, simulated test samples and raw materials, but which test sample to use when conducting a test depends on its function in properly evaluating the safety of the finished product, and it is necessary to be able to demonstrate the scientific justification for that selection. Caution must also be exercised in regard to whether or not there is a chemical change in the raw material or the raw chemical substance during the manufacturing processes (including the sterilization process). If there is no chemical alteration, it may be said that conducting the test on a test sample of the raw material or chemical substance is more pragmatic and reasonable than testing with a test sample from the finished product. The test on the raw material or finished product may also be substituted with a biological safety test on the chemical substance. However, if the manufacturing process chemically alters the raw material or raw chemical substance, testing must be conducted using a test sample taken from the finished product or using a simulated test sample manufactured under the same conditions as the finished product – in the case of a sterile product, this refers to the product after sterilization.

Table 17.5 Necessity for reevaluation in line with changes, etc., to medical device (material) specifications

-
- (a) Change of suppliers or any change in the specifications of the materials used for the product.
 - (b) Any change in the materials, formulation, manufacturing processes, sterilization or primary packaging of finished products.
 - (c) Any change in the finished products during the storage period.
 - (d) Any change in the intended use of the finished products.
 - (e) Any evidence that the product may cause adverse events.
-

In addition, when a new raw material is used or the structural component of an existing medical device or material is changed to a new raw component, the risk to the finished product may also be examined, by conducting a safety test to evaluate that new substance. It is necessary that the items in a safety test on the new raw chemical substance be adjudged as equal to the selection criteria of a test on the finished product. When there are new raw materials, it may be considered a requirement to conduct the necessary and appropriate test and evaluation according to each corresponding test guideline or in accord with OECD guidelines and the Law Concerning the Examination and Regulation of Manufacture, etc., of Chemical Substances, etc.

Iyakushin Notification No.0213001 notes the possibility of the need to implement a biological safety evaluation in any case, listed in Table 17.5. It also requires thorough consideration of the necessity of retesting or adding test items. If the amount of leached substance is negligible from a toxicological standpoint, if toxicity is known and acceptable, or if it is judged to be biologically safe, it is not necessarily required to perform a retest. However, in that case, appropriate examination and/or testing are needed in order to make that determination, so that evidence-based material can be presented.

In terms of their application or of their component material, there are quite a number of types of medical device and each one is unique. Therefore, it is difficult to apply a uniform, common test methodology to all medical devices in order to evaluate their biological safety. Test methodology must be determined by taking into account the principles, sensitivity, selectivity, quantitative capability, and reproducibility of the test, as well as the method of application and the limitations of the test samples with respect to the significance of the biological safety evaluation for the medical device in question. In other words, sufficient justification is necessary for the determination that the obtained results are adequate for evaluating safety in clinical use. It is also essential to clarify the basis and justification when a specific test methodology is selected.

Especially for biological safety tests on medical devices, there are many cases in which it is difficult or even impossible to conduct testing on animals

or using an *in vitro* method with a test sample in the same form as that used by humans. Therefore, a scientifically valid method based on normal usage experience and results is needed in order to secure a test methodology that can evaluate biological safety appropriately.

17.3.1 Cytotoxicity test

Along with a sensitization test, a cytotoxicity test is indispensable for all medical devices, and it occupies an important position in biological safety testing. A cytotoxicity test is an excellent test methodology that can handily detect the toxicity of a chemical substance using an index of apoptosis and morphological change in a substance leached from the medical device and its raw chemical substance. The implementation of a cytotoxicity test is recommended, not only in Iryokiki-Shinsa No.36, but in ISO 10993 and guidelines of various countries as well. The cells utilized in this test include primary cells and established cells obtained from biological tissue. As long as there is no special aim, an established cell line from which constant data can be obtained is often used, regardless of the test facility. Each established cell line has unique features in regard to its form, doubling time, adhesiveness, suspension, and receptivity to chemical substances. Moreover, the cytotoxicity test offers various methods of evaluation, each of which presents specific strengths and limitations, respectively in terms of reproducibility, sensitivity, and simplicity.

As the aim is to evaluate the safety of a medical device, Iryokiki-Shinsa No.36 allows the direct contact method, using the extract itself or using contact with a material from the device, while the colony formation assay is adopted as a method that does not require a special measuring instrument with high detection sensitivity and with which quantitative evaluation is possible. The colony formation assay test method enables the toxic action on the cultured cells of chemical substances to be quantitatively evaluated by counting the number of cell populations (colonies) that have formed from the proliferation of a single cell. That is, the cytotoxicity of a medical device or material is evaluated from test results using an extract from medical devices or material as the test sample. It is also recommended that a colony formation inhibition test be performed using the direct contact method in the case of medical devices employed in the ophthalmologic domain, as well as for medical devices and their materials implanted long term. This involves placing the medical device on the bottom of the culture dish, culturing the cells directly on top of it, and counting the number of colonies formed. It is necessary to exercise caution in the evaluation of this result because, with this method, sensitivity is extremely high due to the cells coming in direct contact with the medical device, and the quality of cellular

adhesiveness on the surface of the medical device also exerts an influence. With the direct contact method, both a substance leached from the material and the effect of the contact between the material surface and the cell can be evaluated. This result becomes a reference for safety evaluation as a supplemental finding to the results of the colony formation assay method that utilizes an extract.

In Iryokiki-Shinsa No.36, the use of one type of negative reference material and two types of positive reference material, each with different toxicity strength, is required in the cytotoxicity test as a standard by which to judge the appropriateness and detection sensitivity of the testing system. A test solution, with which each extract from the medical device and the reference material are diluted to several levels of concentration, is compared with the colony formation rate in a fresh medium, and an extract concentration equal to 50% of the number of colonies of a fresh medium is calculated. A 50% inhibitory concentration of the cell proliferation value (IC_{50} value) is obtained in this way, and the biological safety of the medical device and material is evaluated based on the cytotoxicity of the extract. The IC_{50} value of three types of reference material is important in order to forecast the strength of their toxicity from the IC_{50} value of the tested medical device and material, as well as for confirming the validity of the test system. The description in paragraph '5.6 Correlation between cytotoxicity strength and tissue irritation' under 'Section 1: Cytotoxicity Test' in Iryokiki-Shinsa No.36 serves as reference. With cytotoxicity in which the IC_{50} value obtained is weaker than the positive reference material B (weak positive reference material), slight irritation of the ocular-mucous membrane can occur. With a cytotoxicity between positive reference materials A (strong positive reference material) and B, an inflammatory reaction might occur in the mucous tissue. It is assumed that the possibility of an inflammatory reaction to muscle tissue is high in the case of cytotoxicity that is stronger than positive reference material A. Thus, the use of reference material is assumed to enable the clarification of the difference in receptivity between tissues. Besides the three kinds of reference material, the use of zinc dibutyldithiocarbamate (a substance mixed with positive reference material B) is recommended as a positive reference material in order to clarify the sensitivity and accuracy of the cell. The positive reference material used in cytotoxicity tests serves the purpose of making it possible to compare the difference in toxicity strength between the test methods by (1) assuring the establishment and sensitivity of the testing system, (2) serving as a measure for comparing the level of toxicity of the test substance, and (3) enabling the same reference material to be used even if the applied test methodology changes. Thus it plays an important role. There are various reference materials available, and the Food and Drug Safety Center in Japan also provides both negative and positive reference material. These materials have undergone international

evaluation examination under WG5 in ISO/TC194, and their level of toxicity has been confirmed. They are also listed in ISO 10993-12(ref. 12) as an example of a standard reference material. These reference materials have also undergone a cytotoxicity test five times on the same lot, and a guarantee is appended and distributed only for those that complied with the standard. In addition to the standard material for cytotoxicity tests, short-term intramuscularly implantable negative and positive reference material is similarly available.

Iryokiki-Shinsa No.36 indicates multiple types of cell strains for use in cytotoxicity tests. As noted above, colony formation performance differs depending upon the kinds of cells and culture conditions. It is therefore necessary to confirm that the cells used in the test have good function for colony formation, and that a consistent level of detection sensitivity and accuracy exists. This means that it is necessary to have appropriate background data on each facility that conducts the tests.

When testing with the culture-medium extraction method, an extract is prepared at a ratio of 10 mL of culture medium to 1 g of test sample. When preparing an extract on the basis of the surface area, the ratio should be 1 mL of culture medium per 6 cm².

Iryokiki-Shinsa No.36 used ISO 10993-5¹³(NB, version 2 at that time) and USP 24 (now defunct) as a normative reference, and it can be thought that tests carried out with a test method in general use in foreign countries may also be acceptable. However, this means that, if the appropriate sensitivity and reproducibility are confirmed when the contact tissue of the medical device in question is taken into consideration, it is possible to conduct tests using other methods noted in ISO 10993-5 by simultaneously testing with the above-mentioned positive reference material or the positive reference material adopted in ISO 10993-12. This suggests the necessity of having background data on positive reference material related to the test method to be used. As an important point that should be taken into consideration when selecting a test method, '6. Test Methods' in Iyakushin Notification No.0213001 states, 'In the case of cytotoxicity, ISO 10993-5 "Tests for In Vitro Cytotoxicity" (NB, version 2 at that time) includes the extraction test method (colony method or subconfluent method), the indirect contact method (agar overlay method, filter diffusion method), and the direct contact method (direct contact via the subconfluent method). Since the sensitivity, quantitative capability of these test methods are varied, in order to detect potential hazards for risk evaluation, it is necessary to use a quantitative test method with high detection sensitivity (for example, the extraction test method),' and it can be thought that choosing a test method that uses an extract is given priority. This point of view is also explained as the selection of an appropriate test method in '5. Reference Information' under 'Section 1: Cytotoxicity Test' in Iryokiki-Shinsa No.36.

A cytotoxicity test is a simple test system that can detect toxicity with high sensitivity. The high sensitivity, quantitative colony formation assay is recommended in Japan and, even if cytotoxicity is detected (as long as a risk assessment indicates that it is not excessively strong), it can be presumed that there is a high possibility of it being judged acceptable. On the other hand, the use of the low-sensitivity, non-quantitative agar overlay method and the extraction method, which employs an extract, has been conventionally recommended in the United States. If cytotoxicity is confirmed by this system, it can be surmised that there is a high possibility that the medical device will not comply in terms of safety. Thus, it is necessary to appropriately evaluate the results of the cytotoxicity test on the biological safety of the medical device that is the target of the test only after fully taking into account the differences in each test method.

17.3.2 Sensitization test

A sensitization test evaluates whether or not allergic contact dermatitis, the so-called delayed-type hypersensitivity reaction (type IV allergy), is generated, and to what extent, when a chemical substance is in repeated contact with the body.

The maximization test, Buehler test, and the adjuvant and patch test, etc use guinea pigs and have been conventionally and extensively used as test methods in evaluating sensitization. The maximization method in particular is an outstanding method, and there have been a great many cases of its implementation internationally. Although the maximization method and the domestically developed adjuvant and patch method have been chiefly used for the biological-safety testing of medical devices in Japan, Iryokiki-Shinsa No.36 notes that the evaluation with the maximization method is to be selected. One of the reasons for settling on the maximization method is the fact that it has been said that the sensitivity of the adjuvant and patch method is low in comparison with the maximization method. However, for test samples with which the application of the maximization method is difficult, use of the adjuvant and patch method is still acceptable.

In Iryokiki-Shinsa No.36, the application method for testing is to be selected according to the type of component material in the test sample. In the case of a metal or ceramic, the use of findings on the known sensitization of the metal ions that constitute the test sample is recommended. When there is insufficient data on sensitization, the level of sensitization of the corresponding metal ion solution is to be evaluated. Distilled water or an appropriate alcohol, respectively, should be used in the case of test samples to be dissolved in water or alcohol, and sensitization should be evaluated

with the maximization method. Low molecular organic compounds should be dissolved or distributed uniformly in an appropriate medium that will not affect the judgment of the test results, and a sensitization test conducted using the maximization method. With polymer resins, the fundamental method is, in principle, to evaluate sensitization by using a test solution of an extraction substance obtained using an organic solvent of the highest extraction rate. Therefore, a test that confirms the presence and the amount of extract from the medical device is required prior to conducting a sensitization test. Two kinds of organic solvent, a polar solvent such as methanol and a non-polar solvent such as acetone, are fundamental in confirming this extraction rate. When a sufficient quantity of extraction substance cannot be obtained with these solvents, it is necessary to select other appropriate media. In Iryokiki-Shinsa No.36, both n-hexane and a 1:1 mixture of cyclohexane to 2-propanol are instanced, but there is no clear description with regard to the extraction rate at which to conduct the sensitization test using an extraction substance. An extraction substance is similarly used in genotoxicity tests as well and, as mentioned later, Iryokiki-Shinsa No.36 does differentiate cases where an extraction substance is clearly to be used and where an extract may be used. Though it is in the same guideline, criteria are not specified for the sensitization test. Furthermore, when results already exist of a sensitization test using an extraction solvent other than an organic solvent for a medical device or material with single and temporary contact (less than 24 h), it is possible that risk assessment may be carried out using those results. This description means if there are already results (for example, existing data overseas), and if the clinical conditions for that medical device are for a single use of less than 24 h, those results may be used. However, for cases where there are no such data, it is deemed necessary to conduct evaluation and consideration under a test that utilizes an extraction substance from an organic solvent.

Usually, a medium 10 times the amount of the test sample is used for the confirmation of the extraction rate and the production of the extract. However, for risk assessment when there is a minimal amount of extract or with a relatively small medical device, it is recommended that an extract condensed at least 10 times be used in the test in order to obtain as high a concentration as possible. Depending upon the medical device, for example when a large-sized medical device is used repeatedly, there is also content that can be taken as requiring that testing be conducted with an extract condensed 100 times. It should not be forgotten that, in all of these cases, the confirmation of the extraction rate is important reference data in subsequent risk assessment.

Iryokiki-Shinsa No.36 and ISO 10993-10 (ref. 14) are not necessarily compatible in regard to the medium used for extraction. It can be considered that Iryokiki-Shinsa No.36 requires that the risk of sensitization to human

be known through the consideration of even stricter conditions. '6. Test Methods' in Iyakushin Notification No.0213001 urges caution in selecting the test method for the cytotoxicity test as well as the tests for sensitization and genotoxicity, and states the following consideration: 'Regarding tests for sensitization and genotoxicity, in particular, if the concentration of leached substances in the test solution is low for some extraction solvents, the volume of leached substances used in testing is limited, which may lead to false negative results. The provisions relating to extraction solvents in ISO 10993-12 (NB, version 1 at that time) state that a stressed extraction method must also be considered to detect potential hazards for risk evaluation. To evaluate the toxicity of unknown substances contained in a medical device, a solvent with a high extraction rate must be selected.' It can thus be thought that, in terms of the range of selection, the test method using an extract of a standard physiological saline solution or vegetable oil, etc., noted in ISO 10993, should be placed lower than the second selection in that list.

The third version of ISO 10993-10, which relates to the sensitization test, was unveiled in August of 2010. In addition to the maximization method and the closed-patch test (Buehler test), both of which use guinea pigs, the local lymph node assay (LLNA test), which uses mice, is mentioned there as one of the three main test methods. The first two types are also noted in versions 1 and 2, but the LLNA method was moved from its previous listing in the Annex to the main text with the third edition. The LLNA method is one of the test methods for detecting delayed-type hypersensitivity and is also incorporated in the OECD test method guideline (TG429, 2002). It is also meant to be an alternate test method. There is no indication of the LLNA method in Iryokiki-Shinsa No.36. The LLNA method applies a test sample to the auricle of a mouse, and evaluates whether or not a reaction is induced by its absorption. Therefore, the applicable conditions may not necessarily be fully satisfied by an insoluble extraction substance and, when the results are negative, there is a possibility of a problem as to whether or not the application was appropriate. It can, however, be concluded that the conditions under which sufficient evaluation is possible can be obtained with a raw material of a low molecular weight. However, whether or not data resulting from the LLNA method is acceptable in applications in Japan is unclear, because at this point there are still insufficient results from applications related to medical devices.

There are many cases of an extract from two types of media, a physiological saline solution and vegetable oil, being used as the test sample in the sensitization test noted in ISO 10993-10. A clause on exhaustive extraction is incorporated into the main text of the 3rd edition of ISO 10993-12 (November 2007), and an explanation of the principles for the extraction of samples has been added as Annex C (informative). Iyakushin Notification

No.0213001 and Iryokiki-Shinsa No.36 are listed in the reference data of that 3rd revision. Moreover, especially in Europe, it is rare for a positive reference group to always be established each time at the implementation phase of the sensitization test. It can be said that, according to Iryokiki-Shinsa No.36, calculating the minimum challenge concentration through the establishment of a positive reference group and the establishment of several steps of challenge concentration is indispensable. The idea of establishing the concentration in this way differs somewhat from ISO 10993-10 and ASTM F 720 (ref. 15). Although the meaning and thought behind calculating the minimum challenge concentration are expressed in detail in Iryokiki-Shinsa No.36, the rationale for this is based on a paper by Nakamura *et al.*¹⁶ Since test conditions differ slightly between the European and American test methods and those of Iryokiki-Shinsa No.36, caution is required in implementation.

In addition to the delayed-type hypersensitivity stated above, the necessity of evaluating the effect on the immune system is alluded to in ASTM F 748 (ref. 17). In particular, the need for evaluation is indicated, especially for medical devices using naturally-derived raw material and with a contact period exceeding 30 days. Iryokiki-Shinsa No.36 does not concretely indicate any specific item for evaluation, but '5. Selection of Evaluation Items' in Iyakushin Notification No.0213001 does note that caution is a necessity regarding the effect on the immune system. Various methods exist for confirming the effect on the immune system. When an effect on the immune system is predicted by a systemic toxicity test such as one on subacute toxicity, as well as by a histopathological examination focusing on changes in the circulatory system or on the internal organs of the immune system, etc., further evaluation and examination using the so-called antigenic test may be necessary.

17.3.3 Genotoxicity test

A genotoxicity test uses bacteria, cultured cells, or an animal to detect a substance that has the effect of inducing mutation in a gene or a chromosome. This test investigates whether a chemical substance is a genotoxic agent that acts on a cell's DNA, causes damage, and initiates a gene mutation or chromosomal aberration at the cellular or individual level due to that damage. A genotoxic agent can cause cancer if the damage is generated in the body's somatic cells and, if it occurs in a germ cell or an embryo, there is the possibility of it affecting the next generation as well as the newborn infant. Thus, genotoxicity testing holds an important position as a method for predicting carcinogenicity as well as predicting the possibility of genotoxicity in future generations. When genotoxicity is displayed in a medical device that is used repeatedly, a medical device that is used over the medium to long term, their

material, or their degradation product, etc., it is necessary to avoid their use in consideration of the potential generation of cancer or other genetic effect on subsequent generations.

Iyakushin Notification No.0213001 and ISO 10993-1 require a genotoxicity test for medical devices that are in long-term contact with the body. Unlike pharmaceuticals in which the chemical substance itself is evaluated, medical devices in many cases consist of multiple raw materials, and the assembly of a test method must be conducted with sufficient consideration for the characteristics and usage condition, etc., of each of those materials. Sometimes only a portion of the chemical substances in a raw material is tested and, in many of these cases, an extract and/or an extraction substance from the raw material or finished product is used, so the selection of a solvent and the method of preparing it as a test sample serve as the main points of consideration.

The implementation of two types of tests is the standard requirement in Iryokiki-Shinsa No.36: the reverse mutation test that detects genotoxicity using salmonella and *E. coli* according to guidelines on pharmaceutical toxicity tests, the Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances, and OECD guidelines, and the chromosomal aberration test that detects genotoxicity using cultured mammal cells. Iryokiki-Shinsa No.36 also makes it acceptable to choose the mouse lymphoma-TK test instead of the chromosomal aberration test. Since numerous mouse lymphoma-TK tests are carried out instead of the chromosomal aberration test, in the US especially, it can be assumed that this will contribute to the acceptance of overseas data in Japan. This test is a mutation test that uses a lack of the TK gene (thymidine kinase) as a marker and, because it can also theoretically detect substances that cause chromosomal or numerical aberration, either the chromosomal aberration test or the mouse lymphoma-TK test is being chosen for pharmaceutical testing as well. It can be assumed that this is also consistent with international trends, the Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances, and the OECD guideline. Although, in addition to the reverse mutation and chromosomal aberration tests, ISO 10993-3 (ref. 18) may require a test that investigates the effect on DNA, there is no such stipulation in Iryokiki-Shinsa No.36. However, the need to carry out a test that observes the effect on DNA does arise further in cases where abnormalities are found by the two necessary types of genotoxicity tests. In the case of medical devices, when genotoxic action is suspected through *in vitro* testing, the implementation of a micronucleus test using a mouse is often considered. It may additionally be necessary to consider, for example, a comet assay that looks at DNA-breaking activity, or a transformation test when there is the possibility of the action of a promoter. When carcinogenicity is predicted for a medical device that contacts the body for

a long period of time, investigating carcinogenic possibilities with a transformation test using cultured cells may be contemplated.

Although there is no fundamental difference between the test method itself inside and outside Japan, there is a difference in the method of preparation of the sample used in those tests. In Iryokiki-Shinsa No.36, the preparation of a sample solution for testing is roughly divided into three types according to the attributes of the material. These are, (1) those that do not dissolve in water, but from which an extraction substance can be obtained using an organic solvent, (2) those that do not dissolve in water, and from which an extraction substance cannot be obtained using an organic solvent, and (3) those that dissolve or are suspended in water.

For test samples that do not dissolve or are not suspended in water, the extraction rate is verified using methanol or acetone. When the weight of a medical device is less than 0.5 g and the extraction rate is 1% or more, or the weight is 0.5 g or more and the extraction rate is 0.5% or more, the solvent with the higher extraction rate should be used to acquire the extraction substance to be used in the test. When the extraction rate is less than the above values, the extract for the reverse mutation test should be prepared using dimethylsulfoxide, as though an extraction substance could not be obtained. For a chromosomal aberration test or mouse lymphoma-TK assay, the extracts should be prepared using a culture medium and all testing conducted in regard to these extracts.

For test samples that dissolve or are suspended in water, the samples should be dissolved or suspended in a medium and presented for testing.

In the case of inorganic materials, a determination should be made, as with the sensitization test, based on the data from a genotoxicity test on the compositional metal ions of the corresponding medical device. When those data are insufficient, testing should be on a representative metal ion solution.

For the genotoxicity test, like both the cytotoxicity test and the sensitization test, '6. Test Methods' in Iyakushin Notification No.0213001 urges caution in the selection of the test method. In conducting a genotoxicity test on medical devices that contain a polymer material, guidelines in Japan recommend obtaining an extraction substance by means of an organic solvent and conducting the test on that substance. There is also the point of view that performing extraction using an organic solvent with which there is usually no possibility of exposure might be too severe. On the other hand, the level of detection sensitivity that can be obtained through testing using a culture-medium extract is questionable, and it poses the danger of delivering false negative results. As it is said that no threshold value exists for genotoxicity, there is also the thought that, if genotoxicity is indeed observed, the risk to humans is high even at a very low concentration. Although it is necessary to wait for further discussion in regard to the existence of a threshold value,

or lack thereof, guidelines in Japan are based on the view that testing must be conducted under strict conditions and the possibility of genotoxicity in a material should not be overlooked.

Reverse mutation test using bacteria

A reverse mutation test using bacteria is a simple *in vitro* test using bacteria (amino acid-requiring mutants) that cause mutation in genes in connection with amino acid metabolism. Because amino acid-requiring mutants are unable to synthesize, within the bacteria, the amino acid that is indispensable to their proliferation, they cannot grow in an agar medium in which those amino acids are insufficient. However, if reverse mutation occurs (where the gene connected to amino acid metabolism returns to normal), they can indeed grow in an agar medium and can form a colony. The reverse mutation test estimates mutagenicity by comparing the count of the formed reverse mutation colony to a negative contrast. Generally, a total of five kinds of bacteria are used: four kinds of histidine-requiring *salmonella typhimurium* mutant strains (TA98, TA100, TA1535, and TA1537) and one kind of tryptophan-requiring *Escherichia coli* mutant strain (WP2uvrA). Although the cell line used by a specific test facility may differ, any strain that has a reactivity equivalent to those noted in any guideline is thought to be acceptable.

Chromosomal aberration test using mammalian cells

A chromosomal aberration is roughly divided into structural aberrations, in which the form of the chromosome turns anomalous, and numerical aberrations, in which the number of chromosomes mutates. With a structural aberration, the forms of all of the chromosomes in an individual cell should be observed. Numerical aberrations are also classified into the aneuploidy type, whereby several chromosome numbers fluctuate, and the ploidy type, in which the chromosome number doubles. There are large variations in the chromosome number of cultured cells after long-term passage and, since analysis of the aneuploidy type is difficult, the frequency of ploidy cells, where the chromosome number has doubled, is analyzed.

Mouse lymphoma-TK assay

A mouse lymphoma-TK assay is a test that detects the DNA damage induced by chemical substances with the mutation of the TK gene in mouse lymphoma L5178Y cells used as an index. A major feature of this test is its ability to detect both mutations at a gene level and at a chromosomal level. Its selection as an alternate for the chromosomal aberration test is recognized. It is used instead of the chromosomal aberration test, especially in the US.

Micronucleus test

For medical devices that show a positive result or suspected genotoxic action through *in vitro* testing using bacteria or cultured cells, there is a need to investigate the effect on the whole body (*in vivo*). A micronucleus test using a rodent (a mouse or rat) is the typical *in vivo* genotoxicity test. Since a micronucleus test detects chromosomal segments generated by chromosomal structural anomalies and abnormalities in cell division, as well as micronuclei thought to be the origin of missing chromosomes, the target of testing is fundamentally tissue that displays thriving cell division. Normally, bone marrow or peripheral blood is used. A count of immature erythrocytes with a micronucleus is taken and the induction of micronuclei into the bone marrow cells of the test sample is investigated. The ratio that immature erythrocytes occupy in the total red cell count is also measured at the same time, the proliferation inhibitory action on the bone marrow cells in the test substance is checked, and genotoxicity is evaluated.

17.3.4 Implantation test

With medical devices implanted or placed in the body, the placement sites as well as the durations of use are varied. It is thus necessary to conduct implantation tests for medical devices and their materials that are either implanted in the body or used in a form that connects the inside and outside of the body, and to evaluate the body's reaction. The main tests for this are those that evaluate the kinds of effect that various medical devices and materials implanted in the body's soft tissue have on the tissue around the implant site, as well as those in the case of use in hard tissue. There are times when it may be more beneficial to view the topical reaction of the body, and times when it may be better to view the reaction of the body as a whole.

Generally, the body has a defensive reaction to foreign substances. That reaction changes to a series of processes such as absorption, rejection through phagocytosis, organization, and encapsulation. This starts with the permeation of early stage inflammatory cells, and progresses to granulation formation, and encapsulation, etc. These reactions may go through various modifications, such as being delayed, promoted, or even reinforced, depending upon the type and the components of the medical device or material. An implantation test not only macroscopically observes the effect caused by these test samples, but also aims at histopathological observation as well in evaluating the level of tissue damage from the test sample. Mostly, it is the frequency and extent of the inflammatory reaction exerted on the tissue and the spread of the range of inflammation that is monitored. Evaluation is accomplished by comparing and observing the damage between the test sample and a negative or positive reference material.

The test conditions for implantation tests involves selecting the animal species, the implantation site (such as intramuscularly, intraosseously, or subcutaneously), and the duration of implantation that will be used in the test, based on information that includes the purpose and method of use of the medical device or its material, the component material, and ingredients of the device, its characteristics, and service life, etc. The implantation test described in Iryokiki-Shinsa No.36 is based on a short-term intramuscular implantation test. For medical devices and materials for which the contact period exceeds 30 days, it is necessary to take safety evaluation into consideration through a longer implantation test. In this case, Iryokiki-Shinsa No.36 indicates conducting a test with reference to ISO 10993-6 (ref. 19) ISO 10993-6 also mentions short-term testing like Iryokiki-Shinsa No.36 in the case of non-degradable and non-resorbable material for short-term implantation. However, it also notes that, depending on the case, if it takes from 9 to 12 weeks for an inflammatory reaction to return to normal, it is necessary to observe the long-term reaction for 12 or more weeks in accordance with that situation. It offers a selection table of 12–104 weeks for long-term implantation in rats, guinea pigs, rabbits, dogs, sheep, goats, and swine, but urges careful consideration regarding the necessity of a 104-week test period. The basic principles, including content from the standpoint of animal protection, are expressed in the main text. In addition to the duration of implantation and the tissue reaction in the case of degradable or resorbable material, as well as the test method for subcutaneous, intramuscular or intraosseous implantation, the Annex E notes the method for evaluating inflammatory reaction after implantation as well. A test method in the case of non-solid samples is also indicated. This content is also referred to in Japan when selecting an appropriate implantation site and method for conducting tests according to the application of the medical device. On the other hand, ASTM suggests ASTM F 763 (ref. 20) for short-term implantation tests and ASTM F 981 (ref. 21) for long-term tests. Although, fundamentally, there are no major differences among the three in terms of their testing methods, care should be taken because there is a slight difference in their methods of evaluation.

The short-term intramuscular implantation test indicated in Iryokiki-Shinsa No.36 involves implanting a test sample and a control sample for 7 days and for 28 days inside the paraspinous musculature of a rabbit. Implanting a test sample 10–12 mm in length and 1.0–1.5 mm in diameter is standard, so there is often the need to produce a specimen that differs from the original form of the finished medical device. However, when the test sample is in a form that differs from the finished product, specific and detailed observation and consideration is required when there is a presumption that a reaction might occur in a different part of the body. If it is necessary to conduct the test using an implantation method that differs

from the norm, the validity of that method must be explained. Appropriate evaluation and consideration supported by experience and results with implantation testing on various kinds of medical device is required. In addition, although high-density polyethylene is usually used as a negative reference material in short-term intramuscular implantation tests, the use of SUS 316 is also accepted by Iryokiki-Shinsa No.36 when the test sample is a metal. With histopathological observation, the required observation and evaluation points include the substances that constitute a membrane and their state, fibroblastic hyperplasia, and the infiltration, necrosis, and fatty infiltration of heterophils (neutrophils), lymphocytes, plasmacytes, macrophage, and giant cells. A comparative evaluation with negative reference materials, etc., by measuring the width of the inflammation domain, is called for as well. In addition, although they are often not attached to European and American test reports, caution is required, as Iryokiki-Shinsa No.36 states, that photographs of representative patho-tissue images are usually attached.

A test method should be devised for conducting each implantation test, in line with any differences in the form of the medical device, the quality of its material, the implantation site, and the duration of implantation, etc. With long-term testing, it is also possible to conduct both so-called subacute and subchronic toxicity tests. In that case, it is necessary to continuously observe not only the reaction of the tissue surrounding the implanted sample but also the systemic reaction of the animal, as well as to evaluate biochemical and hematologic tests, etc., including the items required in subacute and subchronic toxicity tests on pharmaceuticals and chemical substances (refer to Section 17.3.6). When conducting these tests, unlike a short-term intramuscular implantation test in which multiple samples are simultaneously implanted in a single animal, only one type of sample may be implanted per animal. Furthermore, the necessity arises of selecting the animal species that is most suited for implantation and performing subacute or subchronic toxicity tests, and for which background data on the various types of test items already exists.

The implantation test described here can also be conducted on test samples for biodegradability and absorbency. In this case, when determining the appropriate test period, it is necessary to evaluate the test sample's *in vitro* decomposition rate and to perform the preliminary testing that is required in order to establish the test period when using a small number of animals (the animals that are actually due to be used for a subacute toxicity test). Moreover, when an implantation test is required within a bone or a blood vessel, it goes without saying that, technically and in terms of appropriate evaluation, an abundance of experience and results are required.

The above test evaluates the histocompatibility of a medical device or material under static conditions, and does not assess mechanical or dynamic

effects or changes in the device or material. Such evaluation should be considered separately, as necessary.

17.3.5 Irritation tests

Section 5 of Iryokiki-Shinsa No.36 relates to irritation tests and includes three different kinds of tests: an intracutaneous reactivity test, a skin irritation test, and an ocular irritation test. These tests are for evaluating the type and level of effect that is caused in regard to tissue damage, inflammation induction, or irritation to the site when a medical device contacts body tissue. The tissue to be used is selected according to the clinical application site of the medical device. It is also necessary to select appropriate test conditions according to the area and method of use of each individual medical device. After cytotoxicity and sensitization tests, an irritation test is the next most demanded test on medical devices or materials. This test is conducted on the skin or applicable membrane, respectively, for medical devices applied to healthy skin or membranes. With a medical device that comes in contact with a damaged surface (skin and membrane) or with blood, it may be concluded that, except in a few cases, an intracutaneous reaction is required.

Irritation tests on locations that are not noted in Iryokiki-Shinsa No.36 should be conducted in accord with ISO 10993-10. In ISO 10993-10, a human skin irritation test is indicated in the main text and Annex C (normative), and an ocular irritation test, oral mucosairritation test, penile irritation test, rectal irritation test, and vaginal irritation test are indicated in Annex B (normative). It can be presumed that test methods in accordance with ASTM F 749 (ref. 22), ASTM F 719 (ref. 23) and other guidelines may also be used. ISO 10993-10 requires the confirmation of the existence of previous data before performing a test that uses an animal, and any such data should be used. When there are no such data, or when those data are insufficient, it recommends considering the possibility of evaluation with an *in vitro* system. An example of a recommended *in vitro* test method that is internationally validated is included in Annex D (informative). It is not certain whether evaluation with an *in vitro* test method would be acceptable in applications for medical devices inside Japan.

In the production of an extract for use in these tests, the extract should be taken by selecting, from the combination of the extraction temperature and duration in Table 17.6, the highest temperature conditions that the test sample can bear according to the ratio of the amount of extraction medium to the test sample as listed in Table 17.7. The temperature must satisfy three conditions: (1) the extraction temperature is lower than the melting point of test sample, (2) the test sample does not degrade under the extraction conditions, and (3) the leached substances do not vaporize or degrade. In

Table 17.6 Extraction conditions for preparing an extract

| Temperature (°C) | Duration (h) |
|------------------|--------------|
| 121 ± 2 | 1 ± 0.2 |
| 70 ± 2 | 24 ± 2 |
| 50 ± 2 | 72 ± 2 |
| 37 ± 1 | 72 ± 2 |
| Room temperature | 72 ± 2 |

Table 17.7 Ratio of extraction medium to test sample when preparing an extract

| Form of test sample | Thickness (mm), material | Amount of test sample per 1 mL extraction solvent (tolerance: within ±10%) | |
|--|--------------------------|--|--|
| Film or sheet | ≤ 0.5 | 6 cm ² | (sum total of surface area (both sides)) |
| | > 0.5 | 3 cm ² | (sum total of surface area (both sides)) |
| Tubing wall | Thickness: < 0.5 | 6 cm ² | (sum total internal and external area) |
| | Thickness: 0.5–1 | 3 cm ² | (sum total internal and external area) |
| Slab, tubing wall, molded product | > 1 | 3 cm ² | (sum total of surface area) |
| Test samples for which surface area measurement is difficult | Elastomer | 0.1 g | |
| | Plastic or other polymer | 0.2 g | |

addition, though it applies to all irritation tests, the pH of the test solution is especially important in the case of an ocular irritation test, and conducting that test is not permitted with a test solution that shows a relatively strong acidity or strong alkalinity (pH 2 or lower or pH 11.5 or higher). In ISO 10993-10, this is clearly indicated for irritation tests using any and all animals.

Intracutaneous reactivity test

The intracutaneous reactivity test is selected for medical devices (or materials) that contact living tissue or body fluid. The extract (extraction solvents: a physiological saline solution or vegetable oil such as sesame oil) from the medical devices is administered into the skin on the back of a rabbit, and the tissue damage or inflammation induction is evaluated. Specifically, the

level of, and recovery from, any topical reaction (erythema, scabbing, edema formation, bleeding, or necrosis) at the site of administration is evaluated. Iryokiki-Shinsa No.36 differs from the methodology in ISO 10993-10 in a few points, such as the number of animals used being slightly larger, not having any provisions for weight at the time of administration, the necessity of documenting with photographs, and the different method of application to the administration site.

Skin irritation test

The skin irritation test aims at confirming whether or not a substance that irritates the skin exists in the extract from a medical device (or materials). The skin's primary state of irritability is a topical inflammatory response produced by a single application of the test substance to a site on the skin. In many cases, that state of irritability does not result in recognizable damage to skin tissue, and is a reversible change. It is also common to record whether the irritability is from a single application or a 24-h application. Change that occurs with continual application to the skin may often be difficult to predict from the results of a primary irritation test, and the need to perform repeated application according to the conditions of use, such as the duration of contact or the repeated use of the medical device (or materials), may arise. It is said that any cumulative skin irritability that develops from this repetitive application is due to accumulation of the irritation-causing substance as well as from the skin's own functional transitions. The skin irritation test is an example of a test in which major differences do not exist inside and outside Japan in terms of the method of implementation and evaluation, and ISO 10993-10 serves as a reference guideline. However, since photographs of representative examples usually need to be attached to applications in Japan, photographs should be attached with the final report.

Ocular irritation test

When a chemical substance is administered via eye drops, an irritative reaction may be produced in the ocular-mucous membrane, due to the physical or chemical irritation. Thus, it is necessary to consider conducting an ocular irritation test for medical devices that may come in contact with ocular tissue. This test evaluates the effect on ocular tissue by applying a test substance and its extract, etc., to the eyes of a rabbit using eye drops. In general, it is necessary to administer eye drops of an extract (extraction solvent: a physiological saline solution or vegetable oil such as sesame oil) from a medical device (or materials) to the eyes of an animal and to evaluate the effect on the ocular tissue in accordance with the Draize or McDonald-Shadduck scoring systems. Although ISO 10993-10 also notes evaluation in

accord with the Draize scoring system, it does not require classification in line with that system through further formula manipulation of the scoring, as Iryokiki-Shinsa No.36 does. As Iryokiki-Shinsa No.36 makes attaching a photograph of the anterior ocular segment attached to the final report when the sum total of the score exceeds six points. Caution is required when using overseas data.

With contact lenses, a test in the form of the so-called ocular study with rabbit eyes may also be necessary. This is in order to satisfy the Contact Lens Approval Standard²⁴ in addition to Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36. The biological requirements for this test are evaluation based on JIS T 0993-1 and the implementation of an ocular study with rabbit eyes as a subacute toxicity test. This test on rabbit eyes references the test method in ISO 9394 (ref. 25). This test is also demanded for conformity to Handling Documents to be Attached to Applications for Approval to Manufacture (Import) Soft Contact Lenses and Antiseptics for Soft Contact Lenses (Iyakushin Notification No.645 (ref. 26)). When a chemical antiseptic is used for sterilization, a cytotoxicity test, sensitization test, and genotoxicity test on the soft contact lens sterilized in line with clinical applications, are required in addition to the test on rabbit eyes. Annex B of ISO 10993-10 also mentions that testing for 8 h a day over 21 days is the exception (a normal ocular irritation test involves an observation period that does not exceed 21 days). In this case, ISO 9394 is referenced for details of the items for evaluation.

17.3.6 Systemic toxicity tests

The body offers various reactions to foreign substances that infiltrate it. For the purpose of comprehensively determining what kind of effect a chemical substance has, or may have, on the body at that time, a systemic toxicity test observes and evaluates the systemic changes on an animal administered with the substance in question. In the content on systemic toxicity testing in Iryokiki-Shinsa No.36, the acute toxicity test (one-time administration), subacute toxicity test (repeated administration for about 1 month), and subchronic toxicity test (repeated administration for about 3 months) are explained in detail. ISO 10993-11 (ref. 27) and ASTM F 750 (ref. 28) are the equivalent standards outside Japan. All of these take such characteristics as the method and duration of use into consideration when selecting the appropriate test method and duration and the items for observation with medical devices.

Acute systemic toxicity test

This is a test to confirm that no chemical substances displaying acute systemic toxicity exist. A single dose of the extract (extraction solvents: physiological

saline solution and vegetable oil such as sesame oil) from the medical device (or materials) is administered into the vein (for physiological saline solution extract) and the abdominal cavity (for vegetable oil extract) of a mouse, and the systemic effects of those test samples are evaluated. The basic test demanded by the guidelines observes the reaction within 72 h after administration. This test mainly checks for the existence of any deviation from the normal state, or cases of death, after administration (observation items are equivalent to those listed in the table in ASTM F 750, except for the evaluation of a concrete change in weight) and confirms whether or not a substance with acute systemic toxicity exists in the extract from the medical device or material. Only in the Japanese guidelines is dissection called for in all cases at the end of the observation period.

Subacute toxicity test (subchronic toxicity test)

This test aims at evaluating the systemic effect on the body and on the contact site, whether through repeated administration (once a day for 4 weeks or more) of the extract from the medical device (or materials) orally, transdermally, or transvenously in line with the actual clinical use of the device, or whether worn or implanted (4 weeks or more in either case) in a form that approximates the actual clinical usage of the medical device. This test is a requisite when the medical device is scheduled for long-term clinical use. Various examinations must be performed and evaluated during the test period as well as at the end of the test. Internationally, ISO 10993-11 is referenced. Within Japan, it is 'Test Methods of New Chemical Substances in the Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances'.²⁹ The inspection items (Table 17.8) provided for in that Law are indicated in Iryokiki-Shinsa No.36.

Fundamentally, an extract of the medical device taken with a physiological saline solution is venously administered daily over 28 days (subacute toxicity test) or 90 days (subchronic toxicity test). Body weight and the amount of food intake are measured at least once per week during the test period, and the state of the animal is monitored daily. Once this test is completed, a hematological exam, serum biochemical exam, pathological anatomy exam, and histopathological exam are conducted as well. The results are integrated and the safety of the test sample is evaluated. Depending upon the device, some may also require an implantation test or a pilot test. If the inspection and observation items required for the systemic toxicity provided for in the Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances are followed and the whole of the body is evaluated, the implantation or pilot test may be replaced with the subacute toxicity test or subchronic toxicity test. However, in that case, unlike the short-term intramuscular implantation test only one test, sample may be evaluated per animal.

Table 17.8 Measurement items in a subacute toxicity test (subchronic toxicity test)

| Item | Frequency or details |
|----------------------------------|--|
| Weight | At least once a week |
| Food intake | At least once a week |
| Hematological examination | Blood coagulation exam including leukocyte count, erythrocyte count, hemoglobin volume, hematocrit value, platelet count, leukocyte percentage, prothrombin time, activated partial thromboplastin time, etc. |
| Serum biochemical examination | Alkali phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase, γ -glutamyl transpeptidase, total protein, albumin, total cholesterol, neutral fat, phospholipids, blood sugar, blood urea nitrogen, creatinine, inorganic phosphorus, Ca, Na, K, Cl, albumin/globulin ratio |
| Pathological anatomy examination | Implemented on all cases and saved for the following organs in accordance with the usual method: brain*, pituitary gland, eyeball, thyroid gland (including parathyroid gland), heart, lungs*, liver*, kidneys*, spleen*, the adrenal gland *, stomach, bladder, bone marrow (femur), testes*, epididymis, seminal vesicle, prostate gland or ovary*, and other organs, and tissue recognized as target organs from macroscopic or other anatomical womb, and other macroscopic findings |
| Histopathological examination | Heart, liver, spleen, kidney, adrenal gland and other organs, and tissue recognized as target organs from macroscopic or other anatomical womb, and other macroscopic findings |

*Measure weight.

In the case of a new raw material chemical substance, not only the test method provided for in the Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances is referenced, but also it is necessary to select and implement an appropriate test method in consideration of various factors, such as the method of use of the medical device and the characteristics of the new raw material chemical substance itself.

17.3.7 Pyrogen test

Pyrogens include endotoxins (toxins derived from gram-negative-bacteria) and non-endotoxic pyrogens (substances derived from microorganisms other than gram-negative-bacteria, or from chemical substances). Iryokiki-Shinsa No.36 mentions the pyrogen test – using a rabbit – that can detect both endotoxins and non-endotoxic pyrogenic substances, and the *in vitro*

endotoxin test, widely used as a method of detecting microbial contamination (endotoxins). The pyrogen test using a rabbit involves administering a physiological saline solution extract of the test substance venously, and determining the existence of pyrogens from the rise in body temperature after administration. The *in vitro* endotoxin test includes the gelation method, which indexes the formation of a gel that occurs via endotoxins derived from gram-negative bacteria acting on a lysate test solution prepared from limulus amoebocyte lysate, and the turbidimetric and colorimetric methods that index optical changes. The test method selected differs according to the purpose of the test (a limit test to check below default values, a quantitative test to check content) and the characteristics (color, existence of reaction interference factors) of the prepared extract. Endotoxins and non-endotoxic pyrogens display both behavior and body temperature raising mechanisms that are complex and indistinct and it is necessary to select extraction conditions according to the properties of the material and the product's purpose of use.

The test method given in Iryokiki-Shinsa No.36 is fundamentally determination criteria equivalent to the pyrogen test method included in the general test methods listed by the Japanese pharmacopeia. It differs slightly from US Pharmacopeia (USP) or European Pharmacopoeia (EP) determination standards. In any case, the test is conducted in consideration of various terms and conditions, such as the content, characteristic, application, etc., of the medical device's raw materials. The test substance or its extract, is administered into the auricular vein of a rabbit, body temperature (rectal temperature) is measured continuously over 3 h, and any difference from the reference body temperature is looked for. When the body temperature (rectal temperature) of at least 2 out of 3 animals rises 0.6°C or more within 3 h post-administration, the result is judged as being positive for pyrogens. Although it cannot be considered as a positive result in and of itself, if one animal shows a body temperature rise of 0.6°C or the sum of the maximum temperature of three animals exceeds 1.4°C , a second test must be performed. Five animals are used for this second test and, if two or more animals then show a body temperature rise of 0.6°C or more, the result is considered positive.

For medical devices that use naturally-derived materials with the possibility of contamination by endotoxins (collagen, gelatin, alginate, etc.), it is preferable for an *in vitro* endotoxin test to be performed as well and the quantity of endotoxins measured. This test and evaluation must be performed for medical devices in contact with circulating blood or implanted in the body.

ISO 10993 contains no parts that address the pyrogen test. This test is, however, noted in Annex F (informative) of ISO 10993-11, not as being indispensable but as requiring a determination on whether it needs to be

performed based on the examination of various factors, such as whether it involves the materials and components of the medical device or a new substance. Just like guidelines in Japan, ASTM F 748 contains a selection table for the corresponding test items. Iryokiki-Shinsa No.36 recognizes that tests conducted under standards such as ISO 10993-11 and USP display a detection sensitivity that is equivalent to domestic test methods.

17.3.8 Blood compatibility tests

A blood compatibility test evaluates whether or not there is a possibility of adsorption and adhesion of blood components, the destruction of blood cells, the coagulation of blood, or the formation of thrombus, etc., or if there is any effect on factors related to the immune function.

A blood compatibility test indicated in detail in Iryokiki-Shinsa No.36 is the hemolytic toxicity test. Although a hemolysis test has been implemented according to the 'method of testing plastic containers for infusion' in the old Japanese pharmacopeia, and which included a fundamental description, it contained no provisions on detailed test conditions and, moreover, was based on visual examination. Furthermore, medical devices without standard criteria required arbitrary judgment. So, in Yakuki Notification No.99, Japan's first unified guideline on medical devices, the test implementation criteria were integrated, beginning with the conditions for preparing the test solution, and according to shape, thickness, material quality, etc. The conception in regard to the method of preparing the extract used in this test is equivalent to that of the irritation test (Tables 17.6 and 17.7). Yakuki Notification No.99 also indicates in detail that the aim is to quantify the test results into a hemolysis rate. The hemolytic toxicity test in Iryokiki-Shinsa No.36 fundamentally follows this same test method. The test methods given in ISO 10993-4 (ref. 30) and ASTM F 756 (ref. 31) are fundamentally the same as those in Japan. Iryokiki-Shinsa No.36 separates medical devices that connect the interior and exterior of the body from medical devices implanted in the body, and indicates examples of evaluation categories and concrete measurement items that are considered to be required respectively for each. These are fundamentally equivalent to the views in ISO 10993-4.

It is necessary to view blood compatibility, other than hemolytic compatibility, according to the usage of the medical device (duration of contact with blood, contact surface area, etc.). In this case, testing is conducted while referencing ISO 10993-4. Since ISO 10993-4 was considered in order to enable the selection of the test items recommended for each classification of medical device, it is preferable to take into account the clinical usage of the medical device when choosing the evaluation items, method, and conditions. ISO 10993-4 is also useful for applications in Japan as a standard for determining the kind of evaluation items to select in regard to blood compatibility.

Depending on the classification of the medical device, something like an intravascular implantation test may also be considered. Although testing over a prolonged period of time also presents very difficult aspects, it may be possible to examine the possible reaction of the body when implanting the medical device intravascularly. It is also possible to explant an implanted medical device and confirm the thrombogenicity of its surface through electron microscope observation, then use the collected blood to measure the number of platelets, and evaluate the blood coagulation examination, hematological examination, and immunological examination (complement system).

It is recommended that blood compatibility tests be implemented by selecting the evaluation items, method, and conditions (*in vivo*, *ex vivo*, *in vitro*) in accordance with the medical device's actual state of clinical use.

17.3.9 Test for supplemental evaluation and other related tests

For medical devices that, over a period of time exceeding 30 days, are used to connect an external communicating device or used implant device, the chronic toxicity test and the carcinogenicity test can be mentioned as items to be used for supplemental evaluation (Table 17.4). In addition, although Iyakushin Notification No.0213001 does not positively specify the usage conditions of the corresponding medical devices, reproductive/developmental toxicity and biodegradation are noted as test items for supplemental evaluation. In regard to the test method to be used for supplemental evaluation of a medical device, no appropriate protocols such as a chronic toxicity test are indicated. If the manual for the guidelines in Yakuki Notification No.99 is followed, just like the evaluation items that are required for safety testing of pharmaceuticals and chemical substances, etc., it may be concluded that the conditions will be met if the observation items and biochemical data, etc., required for the systemic toxicity test are available. Moreover, if those conditions are indeed satisfied, it may be thought that the chronic toxicity test might be replaced by the implantation test or pilot test. Furthermore, when the extracted raw material chemical substance, additive, or degradation product are clear and a marked chronic toxicity can be expected, risk may be assessed based on the chronic toxicity data for those substances. With the carcinogenicity test, because there is no indication of the protocol for conducting and evaluating the appropriate animal testing on the carcinogenicity of the implantation site, a test to evaluate the carcinogenicity of the implantation site is currently not performed. In regard to that carcinogenicity test, as with the chronic toxicity test, when the extracted raw material chemical substance, etc., is clear, and its carcinogenicity is clear, it may be thought important to evaluate the risk using the carcinogenicity data from those substances. Furthermore, a subacute toxicity test, implantation test, and

genotoxicity test are deemed indispensable on the test samples that require supplemental evaluation. If these test results are appropriately examined and evaluated, it could be considered possible to determine quite appropriately whether a chronic toxicity test and carcinogenicity test are needed or not.

In addition, although not especially positively indicated in Iryokiki-Shinsa No.36 as an item for evaluation, reference is made in ASTM F 748 about the necessity of evaluating the effect on the immune system. This point is as mentioned above (see Section 17.3.2). Furthermore, in regard to specific medical devices, care needs to be taken as, in some cases, the required tests and criteria are provided for separately. For example, with contact lenses, there is a test for conformity with antiseptics, an ocular test (see the section on 'Ocular irritation test').

Separate guidelines for dental equipment have been issued as the 'Basic Considerations on the Physical, Chemical and Biological Testing Required for Application to Manufacturing and Distribute Dental Material, Etc.' (Yakushokuki Notification No.0831002) (ref. 32). This was adjusted for consistency with ISO standards, such as ISO 10993 (on medical devices on the whole) and ISO 7405 (ref. 33) (on dental material), and the Japanese JIS standard (Japanese Industrial Standards). It also indicates that medical devices used in dentistry are categorized according to the contact site and the duration of contact and the relationship to the required test items (Table 17.9). For medical devices used in dentistry, implementation of a toxicity test by inhalation is called for through the acute systemic toxicity test and subchronic systemic toxicity test, but when it is judged, based on the risk-analysis method, that the risk of inhalation is acceptable, it is stated that those toxicity tests are not necessary. For example, it can be considered that there are some cases in which risk can be evaluated through oral administration or other pertinent information. In addition to this, it is also stated that a pilot test may also be required for medical devices used in dentistry and that these devices are to be categorized as well (Table 17.10).

With this latest edition to the Pharmaceutical Affairs Law, a third-party certificate system was also introduced in Japan. The medical device must be one for which a certification standard is provided, and it is satisfactory to merely prepare data that conform to that standard. Many of these are also provided for in JIS, and the test method for the required biological-safety test is the same as that mentioned above. Implementation under GLP is not absolutely required.

17.3.10 Confirmation of the extraction rate and production of an extraction substance (extract)

Some finished products for medical devices that include a polymer material, or a polymer material as a component material, add various kinds of chemical

Table 17.9 Guideline for the main evaluation of medical devices used in dentistry

| Categories of medical devices used in dentistry | Duration of contact* | Biological tests | | | | | Irritation/ intracutaneous reaction | Systemic toxicity | | | Genotoxicity | Implanted |
|---|----------------------|------------------|---------------|-------------------|------------|--------------|---|-------------------|--------------|--|--------------|-----------|
| | | Cytotoxicity | Sensitization | Systemic toxicity | | | | | | | | |
| | | | | Acute | Subchronic | | | | | | | |
| | | | | | (Orally) | (Inhalation) | | (Orally) | (Inhalation) | | | |
| | | | | | | | | | | | | |
| Non-contact devices | | | | | | | | | | | | |
| Surface devices | | | | | | | | | | | | |
| Skin | A | ○ | ○ | | ○ | | | | | | | |
| | B | ○ | ○ | | ○ | | | | | | | |
| | C | ○ | ○ | | ○ | | | | | | | |
| Intraoral tissue | A | ○ | ○ | | ○ | | ○ | | | | | |
| | B | ○ | ○ | | ○ | | ○ | | ○ | | | |
| | C | ○ | ○ | | ○ | | ○ | | ○ | | ○ | |
| Breached or surface compromised (damage) | A | ○ | ○ | | ○ | | ○ | | | | | |
| | B | ○ | ○ | | ○ | | ○ | | ○ | | | |
| | C | ○ | ○ | | ○ | | ○ | | ○ | | ○ | |
| External communicating devices | A | ○ | ○ | | ○ | | ○ | | | | | |
| | B | ○ | ○ | | ○ | | ○ | | ○ | | ○ | ○ |
| | C | ○ | ○ | | ○ | | ○ | | ○ | | ○ | ○ |
| Implant devices | A | ○ | ○ | | ○ | | | | | | | |
| | B | ○ | ○ | | ○ | | | | ○ | | ○ | ○ |
| | C | ○ | ○ | | ○ | | | | ○ | | ○ | ○ |

* A: limited contact (within 24 h), B: prolonged contact (1–30 days), C: permanent contact (more than 30 days)

Table 17.10 Guideline for trial-use testing of medical devices used in dentistry

| Categories of medical devices used in dentistry | Duration of contact* | Biological tests | | | |
|---|----------------------|-----------------------------------|-------------------|--------------------------------------|------------------------------------|
| | | Trial-use test on pulp and dentin | Pulp capping test | Trial-use test on root canal filling | Trial-use test on artificial teeth |
| <i>Non-contact devices</i> | | | | | |
| Surface devices | A | | | | |
| | B | | | | |
| | C | | | | |
| External communicating devices | A | ○ | | | |
| | B | ○ | | | |
| | C | ○ | | | |
| Implant devices | A | | ○ | ○ | |
| | B | | ○ | ○ | ○ |
| | C | | ○ | ○ | ○ |

* A: limited contact (within 24 h), B: prolonged contact (1–30 days), C: permanent contact (more than 30 days).

substances of low molecular weight to that polymer material in order to improve processability and formability and to stabilize and maintain performance. There are also some in which a leachable chemical substance of low molecular weight is additionally contained in finished products of complex medical devices that consist of various kinds of composite materials. It is usually predicted that the extraction rate will go up along with an increase in the amount of such low molecular weight substances and an increase in ingredients with a low degree of polymerization in the polymer material. It can be considered that these low molecular weight substances present a high possibility of bringing a greater biological disadvantage than substances of high molecular weight. Thus, as mentioned above, Iryokiki-Shinsa No.36 notes that a problem is especially posed by how much of the extraction substance can be obtained from the medical device, etc., that requires evaluation when conducting the sensitization and genotoxicity tests. This is because the test method must be selected according to the difference in the extraction rate.

As a medium for checking the extraction rate, Iryokiki-Shinsa No.36 mentions methanol and acetone as first choice. As mentioned above, the selection for this medium differs for the sensitization test and the genotoxicity test, with either n-hexane or a 1:1 mixture of cyclohexane to 2-propanol as the second choice with the sensitization test. A further difference between the sensitization test and genotoxicity test is deciding whether to test using an extraction substance or using an extract (Table 17.11). With the genotoxicity

Table 17.11 Example judgment for the extraction rate confirmation test

| Judgment | Weight of medical device | Extraction rate | |
|---|--------------------------|---------------------|-------------------|
| | | Sensitization test* | Genotoxicity test |
| When an extraction substance is obtained | 0.5 g or more | 0.1% or higher | 0.5% or higher |
| | Less than 0.5 g | 0.5% or higher | 1% or higher |
| When an extraction substance cannot be obtained | 0.5 g or more | Less than 0.1% | Less than 0.5% |
| | Less than 0.5 g | Less than 0.5% | Less than 1% |

* As there is no indication in Iryokiki-Shinsa No.36 of the numerical value to be used as criterion, the criterion from Yakuki Notification No.99 was used.

test, when the weight of the medical device is 0.5 g or more and either or both of two kinds of media show a 0.5% or higher extraction rate, two types of genotoxicity test are conducted using the extraction substance obtained through extraction with the medium of the higher extraction rate. When the extraction rate for both media is less than 0.5%, the test is performed with a test solution prepared by extraction with a culture medium. When the weight of the medical device is less than 0.5 g, the extraction rate under this standard is 1%. However, this standard value is not specified with the sensitization test. In addition, any medium with which the medical device deforms or dissolves, to such an extent that it does not retain its original form, can be removed from the choice of media to be used for checking that extraction rate. It is also necessary to investigate the physicochemical relationship between the raw materials that are used in the medical device being evaluated and the extraction medium, and it is desirable that the produced extract or extraction substance be promptly utilized in the test (within 24 h, in principle). When prompt application is difficult, and it is then stored with another extract or extraction substances, or with another medium used in the test, it is necessary to check stability under those conditions. When the test is implemented according to ISO 10993 and ASTM (test solution preparation methods reference ISO 10993-12 and ASTM F 619 (ref. 34)), two kinds of extract, a physiological saline solution and vegetable oil, are generally required, and the viewpoints differ from the Japanese guideline. When these two types of extraction media are used, it is not necessary to confirm the extraction rate.

For materials that clearly do not contain an ingredient that can be extracted by the above-mentioned organic media, for example a medical

device that consists solely of metal and ceramics, there is no need to confirm the extraction rate here either. However, the necessity may arise to consider performing a separate test on leached substances. ISO 10993-14 (ref. 35) and ISO 10993-15 (ref. 36) are noteworthy as reference in that case.

Iryokiki-Shinsa No.36 contains a portion that differs from the use of a physiological saline solution or vegetable oil as an extraction medium as is normal in non-Japanese guidelines, and examples of this causing confusion in regard to the extraction process itself have been seen. An outline is shown in Table 17.12 in order to make the preparation of a solution for testing in accordance with Iryokiki-Shinsa No.36 easier to understand. Fundamentally, except for the cytotoxicity, sensitization, and genotoxicity tests, preparation of the extract is carried out under the same conditions as outside Japan.

17.3.11 Medical devices in the field of regenerative medicine

In the field of regenerative medicine, after adding some kind of processing to cultivate and proliferate the viable cells collected from human tissue, the aim is to return the cells themselves, or in combination with the device, to the human tissue (derived from autologous or heterologous tissue) and aim at recovery of both function and damaged tissue through association with the body. Various types of tissue can be considered for use in regenerative medicine, such as blood, lymphocytes, sperm and ovum, skin, and cartilage, and, in certain cases, as animal-derivative tissue, even the cornea, cardiac valve, and liver can be considered.

Because cultivation and processing of these cells occurs *ex vivo*, examination and confirmation procedures for ensuring safety and quality in each process are required, even in the case of an autograft. The present situation in Japan may call for examination and confirmation of the following kinds of safety for cells used in regenerative medicine: (1) an examination for donor-derived viruses (HBV, HCV, HIV, etc.), in some cases the examination of finished or unfinished products may be necessary, (2) an endotoxin examination, sterilization test, and contamination examination for mycoplasma on raw materials and finished products, (3) the karyotyping of cells (detailed karyotype analysis of changes to the chromosome number at the start and latter half (product) of cultivation, and differential staining of chromosomes) (in addition to chromosomal change during cultivation, the mixing of heterocytes is detectable), (4) a change in the character of processed cells (in order to observe malignant transformation (tumorigenicity) cells, the cells are transplanted subcutaneously in a soft agar culture or a nude mouse, etc., during the latter half of cultivation and a tumorigenicity

Table 17.12 Example test sample preparations for each examination used in biological-safety testing

| Test item | Test sample (test substance) | Extraction medium (test solution) or extract |
|------------------------|---|---|
| Cytotoxicity test | <ol style="list-style-type: none"> 1. Substance that dissolves or is suspended in water 2. Substance that does not dissolve in water | <ol style="list-style-type: none"> 1. Dissolves or is suspended in water or a culture medium 2. Culture-medium extract or direct contact |
| Sensitization test | <ol style="list-style-type: none"> 1. Metal or ceramic 2. Substance that dissolves in water or alcohol 3. Low molecular weight organic compound 4. Composite material or product containing polymer resin | <ol style="list-style-type: none"> 1. Use existing findings 2. Dissolves in distilled water or alcohol 3. Dissolves or is suspended in an appropriate organic solvent 4. Extraction substance (first method*) or extract (second method†) from an organic solvent |
| Genotoxicity test | <ol style="list-style-type: none"> 1. Substance that dissolves or is suspended in water 2. Substance that does not dissolve in water, and from which an extraction substance cannot be obtained using an organic solvent 3. Substance that does not dissolve in water, but from which an extraction substance can be obtained using an organic solvent | <ol style="list-style-type: none"> 1. Dissolves or is suspended in water or a culture medium 2. DMSO extract or culture-medium extract 3. Extract from an organic solvent* |
| Implantation test | Cylinder length: 10–12 mm, width: 1.0–1.5 mm | |
| Irritation test | At the ratio specified in Table 17.7, extraction under the highest temperature conditions that satisfy the conditions in Table 17.10 from the temperature and duration conditions shown in Table 17.6 | Extract of a physiological saline solution or vegetable oil |
| Systemic toxicity test | Same as irritation test | <p>Extraction medium for the acute toxicity test: physiological saline solution and vegetable oil</p> <p>Extraction medium for the subacute (subchronic) toxicity test: physiological saline solution only</p> |

(Continued)

Table 17.12 Continued

| Test item | Test sample (test substance) | Extraction medium (test solution) or extract |
|--------------------------|------------------------------|--|
| Pyrogen test | Same as irritation test | Extract of a physiological saline solution |
| Blood compatibility test | Same as irritation test | Extract of a physiological saline solution |

* Method of obtaining residue from an extract by evaporation of a solvent with a rotary evaporator under as low a temperature as possible.
† Method of condensing or inspissating an extract using a rotary evaporator, etc., and, whether condensing and preparing 1 mL per 1 g of test sample, or dissolving in an appropriate evaporated solvent after solvent evaporation and preparing 1 mL, conducting the test with that as the test solution.

examination performed), and (5) as necessary, a purity test on culture-medium components, and components that are derived from materials or a reagent, a test that shows that the cultivation equipment does not display an adverse effect on the cells, and a feeder cell examination. It can be considered necessary to conduct the same evaluation for culture-medium materials and scaffold materials, etc., as that in the biological-safety test for medical devices. Since some related notices have been released, such as Yakushoku Notification No.0208003 (ref. 37) and Yakushoku Notification No.0912007 (ref. 38), any notice that applies to the device under development should be used as reference.

17.4 Relationship and comparison between the International Organization for Standardization (ISO) standard and American Society for Testing and Materials (ASTM) standard

The targets of biological-safety testing include a large diversity of medical devices. Although the test items required for the risk assessment demanded by ISO 100993-1 and ASTM F 748 differ according to the situation in which each device is used, such as the site of contact between the medical device and the body, and the duration, method and frequency of that contact, the fundamental view is the same as that of Iyakushin Notification No.0213001. ISO 10993-1 was revised to its third edition in August of 2003 and, although it was about half a year after the issue of Iyakushin Notification No.0213001 (February 2003), the selection table for test items presented in ISO 10993-1 at that time was the same as that in ASTM F 748 (the test item selection table was, in general, common

between Japan and Europe up until that point. The fourth edition of ISO 10993-1 was then published in October 2009. At this point, along with a change to content influenced by risk assessment, the table of test items for supplemental evaluation was withdrawn, and, as in ASTM F 748, that information was incorporated into the main text. The selection table of evaluation items in ISO 10993-1 and ASTM F 748 is almost the same as that in Iyakushin Notification No.0213001 (Table 17.1). However, Japan considers its use to be comparatively binding.

During the eight years in which ISO 100993-1 was revised twice, there were no revisions to guidelines in Japan. In the meantime, there were some revisions to other guidelines in the ISO 10993 series, as well as to the ASTM series, and some portions of the information that were quoted in Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36 at that time were changed. However, it can be concluded that, essentially, these changes have had little effect on the interpretation of Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36. Currently, a proposal to revise Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36 is at the stage of examination and preparation. Although it is beyond the realm of supposition to imagine what kind of new guideline will emerge, it can be thought that the fundamental conception in Japan will probably not change. As with the sensitization test and genotoxicity test, the view on testing using an extraction substance obtained with an organic solvent can be considered to be Japan's stance for, in any case, defining safety as the removal of all risks. On the other hand, medical-device safety is tested in foreign countries using an extract under physiological conditions, and it seems that their thought is that safety can also be assured from past results. For the same reason, Japan is considering making risk assessment easier to perform by conducting a quantitative rather than a qualitative test. It seems that they are attempting to ensure safety by improving test reliability and making mutual comparisons easier to perform by using a culture-medium extract, as in the cytotoxicity test, as well as using more than one standard sample. Through that, their aim is to fully grasp both the possibility of toxic manifestation in a material during an evaluation test and the dose-response relationship, with the idea of indicating, as clearly as possible, the level of risk that a medical device or raw material possesses. It might be said that they are adopting a test methodology with higher sensitivity and requiring data based on more severe test conditions. It might also be deemed that, insofar as the requirement in Europe and America is to clarify the level of risk during actual use, there is a fundamental difference to the two views. Although the guidelines in Japan have not been revised in eight years or so, Japan's view on the guidelines for biological-safety testing of medical devices is towards incorporation whenever a portion of the corresponding ISO 10993 series is revised. This will make Japanese data easier to use for other countries.

Considering these differences in the concept of safety, brought about by the diverse historical backgrounds and governments in each country, there can be a large disparity in the regulatory system of each country in regard to medical device safety. Even though the ISO standard was debated and created on an international stage, the posture and level of compliance to that standard, which is reflected in each country's guidelines, differs by country. We have discussed above which of the multiple test methods that exist for a single evaluation item should be selected. Since the way that the extract used in testing is prepared also differs only slightly by country, it is rare for this to become a major problem, but caution is a requisite. Differing character is occasionally evident between the ISO standard, which is seen as an international standard, and the guideline that is binding administratively in each country. As mentioned above, after understanding and taking each difference into account, a biological-safety test is required that is appropriate for the country in which the application for approval will be submitted, naturally including cases of application in more than one country at a time.

17.5 Relationship between classification, examination, and certification in Japan

Medical devices are categorized into three types under Japan's Pharmaceutical Affairs Law:³⁹ 'specially controlled medical devices,' 'controlled medical devices,' and 'general medical devices.' This categorization is determined in consideration of the level of risk related to the site and the duration of contact with the body, as well as any anticipated problems. Even if a device does not correspond as either a 'specified controlled medical device' or a 'designated controlled medical device,' it is categorized into either 'specially controlled medical devices,' 'controlled medical devices,' or 'general medical devices' and this fundamental categorization is called 'classification.' Although there are three classifications by law, there are four in a specific notice (Yakushoku Notification No.0720022 (ref. 40)). Classes III and IV correspond to specially controlled medical devices, Class II relates to controlled medical devices, and Class I is for general medical devices. The three classifications are based on the perspective of whether approval under the Pharmaceutical Affairs Law is required or not, and whether third-party certification is required or not. Those that require approval under the Pharmaceutical Affairs Law are placed into Classes III and IV, and those are further differentiated, based on the method with which the approval review is advanced. Which Class a medical device falls under is clear and understandable by referring to Yakushoku Notification No.0720022. In general, though, medical devices implanted for use inside the body are in Class IV (specially controlled medical devices) and those that, during use, directly contact the inside of a blood vessel or the mucosa, or that implement

treatment by applying high-level energy to the body are placed in Class III (specially controlled medical devices). Those with a low level of risk are designated for Class II (controlled medical devices) and those with a very low level of risk are in Class I (general medical devices). In cases where one item includes multiple generic names and there are no generic names that infer a multifunction device, the generic name for the device with the highest risk classification should be selected. When more than one device is in the highest risk classification, the generic name is mostly selected based on the principal performance of the device. The relationship between approval and certification based on the revised Pharmaceutical Affairs Law is indicated in Table 17.13.

Table 17.13 Overview of medical-device classification and Pharmaceutical Affairs Law regulations* (approval and certification)

| Class | Medical device classification by risk | Generic name (no. of listings*) | Manufacture and distribute | | Distributor/lessor |
|-------|--|---|----------------------------|---|------------------------------------|
| | | | Permission | Approval | |
| IV | Devices that are highly invasive to patients and that can present a danger to life if a malfunction occurs | Specially controlled medical device 332 | Required | Approval required | Permission required |
| III | Devices that can greatly affect the body if a malfunction occurs | Specially controlled medical device 749 | Required | Approval required | Permission required |
| II | Devices that present a low possibility of danger to life or serious functional problem | Controlled medical device 1789 | Required | Approval or certification [†] required | Notification required [‡] |
| I | Devices that would have a minor effect on the body even if a malfunction occurs | General medical device 1195 | Required | Not required (notification required/self-certification) | Nothing in particular [‡] |

*As of March 31, 2011.

[†]Certification is limited to specific controlled medical devices.

[‡]For specified controlled medical devices, even if categorized under Class I or II, the distributor and lessor must be licensed.

17.6 Outline of the medical device Good Laboratory Practice (GLP)

The Medical Device GLP was issued in March of 2005 as Ministry of Health, Labor and Welfare Ordinance No.37, and partially revised in a June 2008 revision (Ministry of Health, Labor and Welfare Ordinance No.115 (ref. 41)). Prior to the Medical Device GLP, the GLP system was fully applied to biological-safety testing of medical devices with the Medical Instrument GLP, issued in September 2002. Before that, whenever GLP testing of a medical device was required, it was carried out in compliance with the regulations in the GLP on pharmaceuticals (Ministry of Health and Welfare Ordinance No.21, Ordinance for the partial revision of the GLP on pharmaceuticals (Ministry of Health, Labor and Welfare Ordinance No.114 (ref. 42))) or the GLP on chemical substances (Law Concerning the Examination and Regulation of Manufacture, etc., of Chemical Substances).⁴³

A typical item of importance in the revision of June 2008 aimed at international compatibility, and it did so through the incorporation of OECD-GLP principles and the integration of much of the content of OECD documents. In line with the GLP Compliance Inspection Organization Local Evaluation System agreed to in 2006, the Pharmaceuticals and Medical Devices Agency and the Ministry of Health, Labor and Welfare became the world's first target of evaluation by the OECD using that system in September of 2008.

We would hope that the implementation and enhancement of an appropriate biological-safety test according to the domestically – as well as internationally – respected Medical Device GLP will be achieved.

17.7 Conclusion

Biological-safety testing for medical devices in Japan refers to Iyakushin Notification No.0213001 and Iryokiki-Shinsa No.36 for the indicators and test methods of fundamental safety evaluation, and they are conducted in adherence to the Medical Device GLP. The fact that, due to this, safer medical devices that have received the appropriate evaluation will be used within Japan, as well as internationally, is encouraging. Because the indicators for the biological-safety tests that need to be implemented and the corresponding method of evaluation are provided for by the world's superpowers, the sure implementation of various safety tests for new medical devices has become simpler. In line with technological advancements and changes in the structure of diseases, enhanced functionality and greater diversity are aimed at in medical devices and it can be assumed that they will play an even more important role in medical treatment in the future. These devices will also answer the need for diversification at medical institutions, as well as for improvement in Quality of Life. In order to meet various new demands

as well, it can be thought that the development and application of new materials will advance further, and that there will be a continued increase in the number of new medical devices, as well as in those with a complex or advanced composition that combine multiple materials. It is also indispensable to deliver outstanding products to worldwide medical treatment sites more rapidly. Thus, a concentration of interdisciplinary knowledge is increasingly demanded in regard to the implementation of biological-safety testing for medical devices and materials of which many are emerging as complex systems.

Yet, internationalization in the field of medical devices actually compares favorably with other fields. However, along with the increase in export and import activity, there is a need for tests that follow the guidelines of foreign countries, in addition to ISO standards, and a need to examine additional tests for application within Japan. It cannot necessarily be said that there has been sufficient international cooperation in the various decisions made in this field. There are cases in which it is necessary not only to sufficiently understand the test method guidelines of the corresponding country, but also to implement testing itself according to ISO standards or to individual guidelines in accordance with the situation of the country in which application will be made. From now on, while promoting further international cooperation that incorporates the ISO standard, it is important to assert what must be asserted in order to ensure safety, and to aim at user safety and convenience. Just as international uniformity of test method guidelines is advancing for pharmaceuticals and new chemical substances through progress in international cooperation, it is also necessary to pay attention to the aim for international unity in test method guidelines for medical devices. By accepting GLP-compliant data in many countries, duplicate testing, including unnecessary animal tests, can also be avoided. Further advancement in international cooperation is desired in GLP and test method guidelines related to the biological-safety testing of medical devices based on sufficient consideration and assurance of user safety.

Under such circumstances, it is necessary for those who conduct biological-safety tests to accurately grasp such trends in the future and aggressively conduct those tests in order to aim at safety for medical-device users. Depending on the device, the need to develop new and appropriate test methods, rather than remain with those conventionally used, will surely arise. Biological-safety tests differ in terms of the accuracy and sensitivity required, as well as the scale of the test, depending upon the various conditions under which the test is conducted (such as the type of medical device or material and the purpose of implementing the test). Since a single test method cannot be applied to all cases, it is important to select the test method that is appropriate for each situation, and it is necessary to support the appropriate judgment in that regard. Those connected with the realm of

biological safety in medical devices must never forget to focus on the necessary knowledge, and the refinement and enhancements of techniques that are required in order to ensure safety.

17.8 References

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Abstract: This chapter describes some interpretations of ISO 10993 and the additional requirements of the Chinese State Food and Drug Administration (SFDA). Guidelines are provided for specific testing, along with a description of the tests required for one example device. The chapter ends with an overview of the bodies responsible for medical device regulation in China.

Key words: ISO 10993, biocompatibility, Chinese State Food and Drug Administration (SFDA).

18.1 Introduction

Since China joined the World Trade Organization (WTO), an increasing number of foreign medical devices have come onto the Chinese market. As a result, an understanding of the regulations relating to the biological evaluation of medical devices in China has become critical for foreign medical inspectors and supervisors. This chapter outlines some interpretations of the ISO 10993 standard, and describes the additional requirements imposed by the Chinese State Food and Drug Administration (SFDA), as well as providing some examples of the regulation in use.

18.2 Interpretation of ISO 10993 and additional State Food and Drug Administration (SFDA) requirements

Biological evaluation of medical devices was performed according to ISO 10993 standards in China. At the same time, some particular requirements were taken into account by SFDA.

18.2.1 General testing guidelines

Chinese assessors adhere strictly to the requirements of the ISO standard. The biological evaluation of medical devices takes into account the nature and mobility of the chemical constituents in the materials used to

manufacture the device, but also considers other non-clinical tests, clinical studies, and post-market experiences to develop an overall assessment. The selection of the appropriate *in vitro* or *in vivo* tests is determined on the basis of the end-use applications of the product: *in vitro* screening should be undertaken prior to commencement of *in vivo* testing. All tests should be conducted according to Good Laboratory Practice (GLP).

18.2.2 Guidelines for specific tests

In some tests of biological evaluation of medical devices, SFDA has its own explanations.

Genotoxicity

‘GB/T16886.3-2008 Biological evaluation of medical devices – Part 3’ deals with tests for genotoxicity, carcinogenicity, and reproductive toxicity, and requires genotoxicity testing to be performed on the basis of an initial decision to test in accordance with either Option 1 or Option 2, described below:

Option 1

- (a) test for gene mutations in bacteria (OECD471);
- (b) test for gene mutations in mammalian cells (OECD476); and
- (c) test for clastogenicity in mammalian cells (OECD473).

Option 2

- (a) test for gene mutations in bacteria (OECD471); and
- (b) test for gene mutations in mammalian cells (OECD476), specifically a mouse-lymphoma assay incorporating colony number and size determination in order to cover both endpoints (clastogenicity and gene mutations).

Hematology

‘GB/T16886.4-2003 Biological evaluation of medical devices – Part 4’ requires that certain devices in contact with blood must be evaluated by a number of different hematology testing methods. Such devices include intravascular catheters, guidewires, intravascular stents, artificial vessels, and implantable administration devices.

Cytotoxicity

‘GB/T16886.5-2003 Biological evaluation of medical devices – Part 5’ recommends that the cytotoxicity of the final product should be evaluated by testing extracts and through direct contact testing.

Implantation periods and observation phases

‘GB/T16886.6-1997 Biological evaluation of medical devices – Part 6’ addresses issues relating to local effects after implantation, and prescribes a test period of no less than 12 weeks for implantation in muscle and subcutaneous tissue, and no less than 26 weeks for implantation in bone. The test period should be the degradation time of the degradable/resorbable material, and several observation phases should be chosen in order to observe the responses of the tissue surrounding the test materials. Changes may be observed in the implantation during the degradation phase degradable/resorbable material; the observation period should therefore be longer than the degradation period.

Registration of interventional products and affiliated devices

The chemical capability and biocompatibility of interventional products and affiliated devices, such as stents, catheters, and guidewires, must be tested separately for registration purposes. This is due to their different behavior in contact and/or the differences in time spent in contact with human tissue.

18.2.3 Guidelines for drug containing devices

The term ‘drug containing devices’ refers to products of which the major function is as a medical device, but in which that function is assisted by a drug contained within it, or in which a drug provides the device with an additional function.

Examples of such devices in China are complex catheters or stents. Within the SFDA, the Department of Medical Devices is responsible for regulating medical devices, while the Department of Drug Registration is responsible for regulating drugs. However, any drug used with a medical device has a different release and delivery route (i.e. it is not used alone). The unique release route is associated with the combining device; the examination and evaluation of the drug(s) used, therefore, cannot be separated from the evaluation of the device itself. Thus, drug containing devices must be registered as medical devices, and come under the jurisdiction of the Department of Medical Devices, since their major function is as devices and any drugs contained within them are not used alone.

The principal considerations in the evaluation of a drug containing device are:

- (a) Regular device evaluation requirements;
- (b) Regular drug evaluation requirements;
- (c) Special requirements concerning the rationality of the drug–device combination mechanism, including unknown reactions and unwanted effects, etc.

18.2.4 Example of biological evaluation of a medical device

This section describes the tests applied to a bioabsorbable vascular scaffold (containing transportation system and scaffold), as required by the SFDA.

The transportation system and scaffold must be evaluated separately, because the former is the external communicating device and the latter is the implant device.

The following tests must be carried out on the transportation system:

- (a) pyrogen,
- (b) hematology,
- (c) systemic toxicity (acute),
- (d) *in vitro* cytotoxicity,
- (e) intracutaneous reactivity,
- (f) delayed-type hypersensitivity,
- (g) hemocompatibility: thrombosis and coagulation.

Many of the same tests are carried out on the scaffold, but additional testing is also required. The full list is as follows:

- (a) pyrogen,
- (b) hematology,
- (c) systemic toxicity (acute),
- (d) *in vitro* cytotoxicity,
- (e) intracutaneous reactivity,
- (f) delayed-type hypersensitivity,
- (g) hemocompatibility: thrombosis,
- (h) genotoxicity.
- (i) Ames Mouse-lymphoma assay and *in vitro* mammalian chromosome aberration test,
- (j) implantation in muscle (test period not less than 12 weeks including inspecting phase 2 weeks/4 weeks/12 weeks),
- (k) *in vitro* degradation,
- (l) subchronic toxicity.

18.3 Major professional bodies

Major professional bodies related to supervision and administration of medical devices in China include Department of Supervision, Technological Evaluation Center and Quality Inspection Center.

18.3.1 Department of Medical Device Supervision, SFDA

The main purposes and functions of the Department of Medical Device Supervision, as provided on the SFDA website (<http://eng.sfda.gov.cn>) are:

To organize the formulation of national medical device standards and supervise their implementation; to draw up the classification list of medical devices; to take charge of registration and regulation of medical devices; to draw up good practices for clinical trials, production and distribution of medical devices, and supervise their implementation; to take charge of the supervision of licensing of medical device production and distribution; to organize the adverse events monitoring, and medical device reevaluation and elimination.

<http://eng.sfda.gov.cn/WS03/CL0790/>

18.3.2 Center for Technological Evaluation of Medical Devices, SFDA

The Center for Technological Evaluation of Medical Devices (<http://www.cmde.org.cn>), part of the SFDA, is responsible for: the technological evaluation and testing of imported medical devices and new medical device products; technological review of registration for trial production and approved production of domestic Class III medical device products; and the review of clinical trial protocols.

18.3.3 Jinan Quality Supervision and Inspection Center for Medical Devices, SFDA

Authorized by the SFDA, the Jinan Quality Supervision and Inspection Center for Medical Devices (<http://www.sdjczx.com/English/index.html>) is a nationally recognized third-party inspection laboratory working in the field of biological, chemical, physical, mechanical, and electrical inspection for a wide range of devices and products, including medical polymer products, biological materials, pharmaceutical packages, physiotherapeutic devices, etc. The laboratory employs unique testing techniques, particularly for medical polymer products and biological materials, such as transfusion, infusion, and injection equipment. The Center has excellent testing facilities and experienced testing staff, and is also the unit to which the Secretariats of the China National Standards Technical Committee of Infusion Equipment for Medical Use and the China National Standards Technical Committee of Biological Evaluation of Medical Devices are allocated.

Table 18.1 Quality supervision and inspection centers for medical devices in China

| Facility | Web address | Speciality |
|---|---|-------------------------------------|
| Center for Medical Devices Testing of National Institutes for Food and Drug Control | http://www.nicbpb.org.cn/en | Tissue engineered materials |
| Beijing Quality Supervision and Inspection Center for Medical Devices | http://www.bimt.cn/ | <i>In vitro</i> diagnostic reagents |
| Peking University Quality Supervision and Inspection Center for Medical Devices | http://www.pku-dmdtc.com/ | Oral cavity material |
| Tianjin Quality Supervision and Inspection Center for Medical Devices | http://www.mdtc.org.cn | Orthopaedic devices |
| Shenyang Quality Supervision and Inspection Center for Medical Devices | http://www.lmti.cn/ | Radiation instruments |
| Hubei Quality Supervision and Inspection Center for Medical Devices | http://www.whmit.cn/ | Medical ultrasound equipment |
| Shanghai Quality Supervision and Inspection Center for Medical Devices | http://www.cmtc.com.cn/TyanWeb/SHYL/index.aspx | Electrophysiological instruments |
| Hangzhou Quality Supervision and Inspection Center for Medical Devices | http://www.shmt.com.c\n/Index.htm | Optics |
| Guangdong Quality Supervision and Inspection Center for Medical Devices | http://www.gdmit.cn/index_en.asp | Cardiopulmonary bypass equipment |

18.3.4 Other quality supervision and inspection centers for medical devices within the SFDA

Table 18.1 presents the other facilities in China responsible for quality supervision and inspection of medical devices (all within the SFDA).

18.4 References

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Microscopic and ultrastructural pathology in medical devices

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Abstract: Microscopic and ultrastructural analyses of animal tissues from non-clinical studies are conducted to document the safety (biocompatibility), performance (function), and efficacy (capacity to treat or prevent disease) of medical devices. This chapter presents a brief overview of basic principles and techniques, as well as innovative methods that are applicable to microscopic and ultrastructural evaluations of biomaterial and medical devices. Illustrative examples are presented throughout the chapter. Basic tissue responses and microscopic evaluation of local and systemic effects in safety studies are discussed. Macroscopic, submacroscopic, and microscopic techniques for assessment of biomaterial performance are presented. One section is devoted to describing semi-quantitative microscopic and quantitative histomorphometric techniques and their application in the evaluation of biomaterials and medical devices. Ultrastructural evaluation of cellular, subcellular, and extracellular changes using both scanning and transmission electron microscopy are discussed. The chapter concludes with a section describing the various factors involved in the morphologic assessment of ocular devices, including the animal model, results of ophthalmic examinations, and preparation of globes for microscopic examination. This chapter attempts to be informative and to articulate the importance of microscopic and ultrastructural pathology in determining the safety and performance of the wide variety of medical devices.

Key words: histopathology of medical devices, safety and performance evaluation, processing and sectioning of specimens, ultrastructural pathology, ocular pathology.

19.1 Introduction

Microscopic and ultrastructural analyses are conducted to document the safety (biocompatibility), the performance (function), and efficacy (capacity to treat or prevent disease) of new implants at tissue and cellular levels. Implants are defined here as biomaterials, medical devices, or combination products (products combined with drugs or their active substances) surgically placed within the animal or human body.¹ Microscopic evaluation of safety has a central position in regulatory testing programs (ISO 10993,

ASTM International, EMEA guidance documents, FDA recommendations).²⁻⁹ The standard ISO 10993 – Part 6 is specific to the testing and evaluation of local effects on soft or hard tissues in contact with an implant,⁷ whereas the systemic effects of implants are covered by the ISO 10993 standard – Part 11.⁸

For both safety and performance, non-clinical and clinical evaluation of implants requires the examiner to have a basic background and understanding of materials science, bioengineering and pathobiology.

Although microscopic and ultrastructural investigations are used routinely in the assessment of implants, specific techniques may be needed to help with the understanding of the implant or host response. These techniques include:

1. The use of imaging techniques, performed in-life or following euthanasia based on X-ray or Doppler ultrasound imaging to allow for a continuous follow-up of the behavior of the implant or host response.
2. The development of quantitative criteria to characterize the descriptive pathology.
3. The integration of computer-assisted analysis, allowing image analysis using morphometric criteria, large fields reconstitution, and statistical comparisons of objective criteria.

The understanding of the complex reactions involving specific implant chemical, physical and thermal characteristics, as well as cellular and secretory factors, collectively determines success or failure of medical devices.¹⁰ Understanding the molecular, cellular, tissue, and organ pathobiology, as well as the principles of healing, are essential for evaluation of the local implant or tissue interface and potential systemic effects.¹¹ Selection of the animal species, surgical technique, healing time, clinical endpoint, technique for specimen preparation, and finally morphologic (microscopic and ultrastructural) criteria are pivotal for the evaluation of the implant or host response. As discussed in this chapter, these criteria have to be adapted specifically for each device or implant.

As for the full testing program of biocompatibility, it is advised to conduct these microscopic and ultrastructural investigations under Good Laboratory Practices or under an ISO 17025 quality system to ensure competent, reliable, and independent analysis of the implant/host response.

The safety assessment of medical devices is complicated by the diversity of devices and components, but also by the variety of clinical indications for these devices. The purpose of this chapter is not to exhaustively deal with all microscopic and ultrastructural evaluation methods for implanted medical devices, but rather to discuss commonly used and innovative methods for their investigation and interpretation of the implant or host response.

Most of the examples mentioned in this chapter are taken from actual studies performed at NAMSA or Biomatech. Specific implant identifications are not disclosed for confidential reasons. Local and systemic safety assessment, implant performance, and quantitative and ultrastructural pathology safety, as well as technical aspects regarding specimen preparation are addressed in this chapter. A specific subchapter discussing ocular medical device testing and evaluation is included as an example of the multiple parameters that have to be considered for each specific implantation site.

19.2 Morphologic assessment in the safety studies of biomaterials and medical devices

Morphologic evaluation, both macroscopic and microscopic, provides essential information regarding biological responses to a medical device or biomaterial. The use of carefully selected animal models for evaluation of tissue responses to biomaterials is critical for non-clinical assessment of both performance and safety. This section focuses on the evaluation of morphologic endpoints relative to safety assessment, rather than on performance or efficacy. Light microscopic evaluation of tissue sections is still one of the most important and widely used techniques for defining tissue responses to biomaterials, both at the tissue/implant interface (local effects) and in distant tissues or organs (systemic effects).

This section will provide an overview of local microscopic changes associated with the implantation or injection of biomaterials into soft tissue (typically subcutis or skeletal muscle). Additional information is presented regarding microscopic tissue changes associated with systemic effects following biomaterial implantation or injection or parenteral administration of biomaterial extracts.

19.2.1 General principles

Specific recommendations for animal selection and study design are detailed in various regulatory and guidance documents.^{7,8,12} Every effort should be made to mimic the clinical use of the test device or biomaterial.

For example, vascular devices should be tested using intravascular implantation, materials intended for mucosal application should be tested using mucosal (oral, rectal, vaginal) exposure, and materials or devices for intra-abdominal use should be implanted to allow the evaluation of peritoneal or serosal reactions. In specific instances, it may be appropriate to stress the test system to simulate adverse clinical observations and, for example, it may be appropriate to use skin-wound models for testing products intended for topical application, thereby evaluating effects on dermal and subcutaneous tissue should the epidermal barrier be breached.

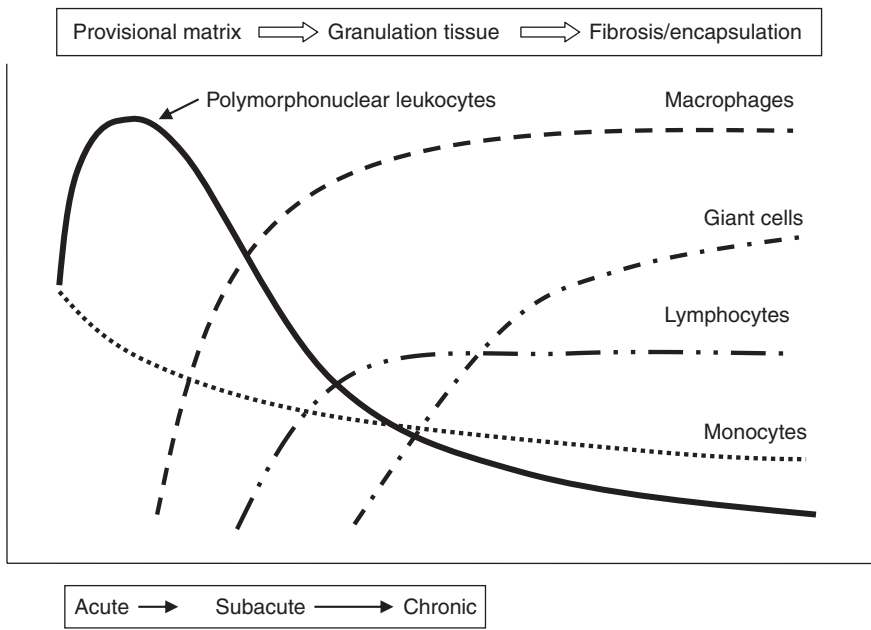
Tissues require proper handling and fixation to provide optimal specimens for microscopic evaluation. Tissues should be removed by trained prosectors as soon as practicable after death or euthanasia. Regarding the histological treatment of harvested organs, it is recommended to follow the guidelines proposed by the Registry of Industrial Toxicology Animal (RITA) procedures.^{13–15} The intent of the RITA procedures is to provide standardized sectioning of tissues and to optimize the comparability and interpretation of the biological responses across the treatment groups and among laboratories.

Prosectors should be trained in proper tissue handling to minimize traumatic damage or desiccation, and organs designated for weighing should be kept moist with 0.9% saline solution. All specimens should be placed in fixative as quickly as possible to minimize autolysis. Morphologic evaluation for local tissue effects requires carefully harvested specimens without the disruption of the tissue/implant interface. In general, the use of tissues for mechanical testing (e.g. tensile strength, burst pressure, or the removal of test articles for ancillary testing) will disrupt the tissue/implant interface and/or alter tissue morphology. If ancillary testing is required, studies should be designed to provide additional intact and undisturbed implant or injection sites for microscopic evaluation. The preparation of implant or injection sites for microscopic evaluation is dependent on the density and hardness of the implanted material. Dense or hard materials will likely require plastic embedding for sectioning or preparation by grinding (see Section 19.4 on specimen processing and Fig. 19.12).

Hematoxylin and eosin staining of implant or injection sites is usually adequate for the evaluation of local cellular and tissue responses. Specialized staining techniques are useful in further differentiating collagen (trichrome stains) or enumerating inflammatory cell subpopulations (immunohistochemical stains, see Section 19.5 on staining recommendations). Generally, soft tissues embedded in paraffin and stained using hematoxylin and eosin are suitable for morphologic evaluation in screening for systemic effects.

19.2.2 Morphologic assessment of local effects

The implantation or injection of a biomaterial or medical device results in physical injury to local tissues, with attendant cellular and tissue responses. Responses are dependent on many variables which include: size of implanted material, extent of surgical trauma, rigidity or surface complexity of implanted material, viscosity of injected materials. In spite of these complexities, there are basic common sequential tissue and cellular responses at play (Fig. 19.1).¹⁶ Initially, there is some degree of local hemorrhage and exudation of plasma proteins with the formation of a provisional fibrin



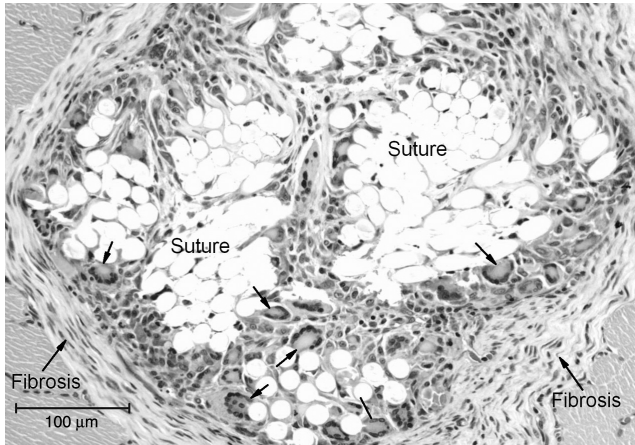
19.1 Temporal sequence of local cellular and tissue responses following soft tissue implantation/injection of biomaterials. (Source: Adapted from Anderson, 2001.)

matrix. Provisional matrix formation is accompanied by the infiltration of polymorphonuclear leukocytes and monocytes. Monocytes become tissue macrophages and function to clear local tissue debris. The provisional matrix provides a scaffold for fibroplasia and neovascularization with subsequent formation of granulation tissue.

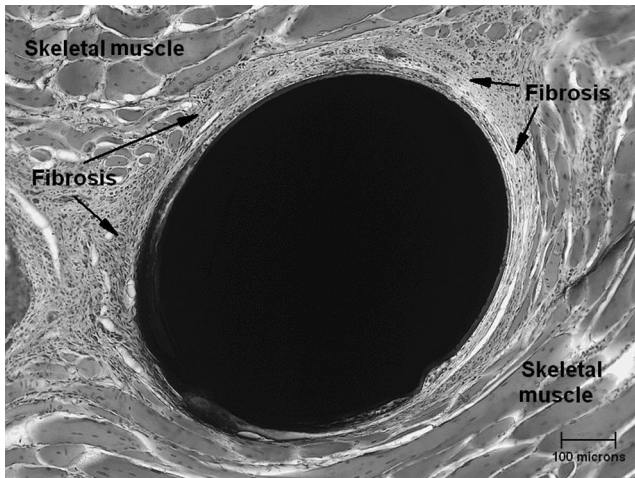
At the same time, there is limited infiltration of lymphocytes, consistent with a non-specific inflammatory response. If local tissue damage is limited and temporal, the surrounding granulation tissue will organize and form a fibrous capsule.

Depending on the persistence and surface features of the implanted or injected material, there may be continued stimulus for monocyte or macrophage recruitment and associated formation of multinucleated (foreign body) giant cells. These cells, formed through the fusion of macrophages, are typically associated with materials having a high surface area to volume ratio, such as surgical meshes, sutures, tissue scaffolds, vascular grafts, and stents (Fig. 19.2).

Durable smooth-surfaced materials will typically elicit a limited macrophage and giant cell response, and will be surrounded by a well-formed fibrous capsule (Fig. 19.3). It should be noted that degradable biomaterials



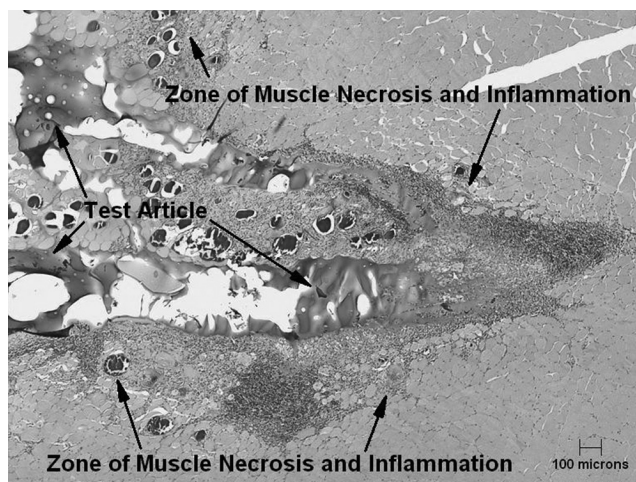
19.2 Macrophage and multinucleated giant cell (arrows) response to suture material with high surface area to volume ratio.



19.3 Fibrosis with minimal cellular reaction surrounding metal device implanted into skeletal muscle. Plastic tissue section stained with hematoxylin and eosin after grinding.

may elicit striking, but transient, inflammation and foreign body reactions as part of the degradation process.

Expanded zones of tissue necrosis with or without inflammation at implant or injection sites are considered adverse (Fig. 19.4). Adverse local cellular or tissue effects may be due to toxicity or immunological responses



19.4 Adverse local reaction with expanding zone of skeletal muscle necrosis and inflammation surrounding test article.

associated with the intact test article*, test article degradation products, or leachable components.

Cross-species (xenogenic) immunological reactions are common with materials of biological origin, such as collagen-based products, and are typified by a lymphocytic inflammatory response (Fig. 19.5).

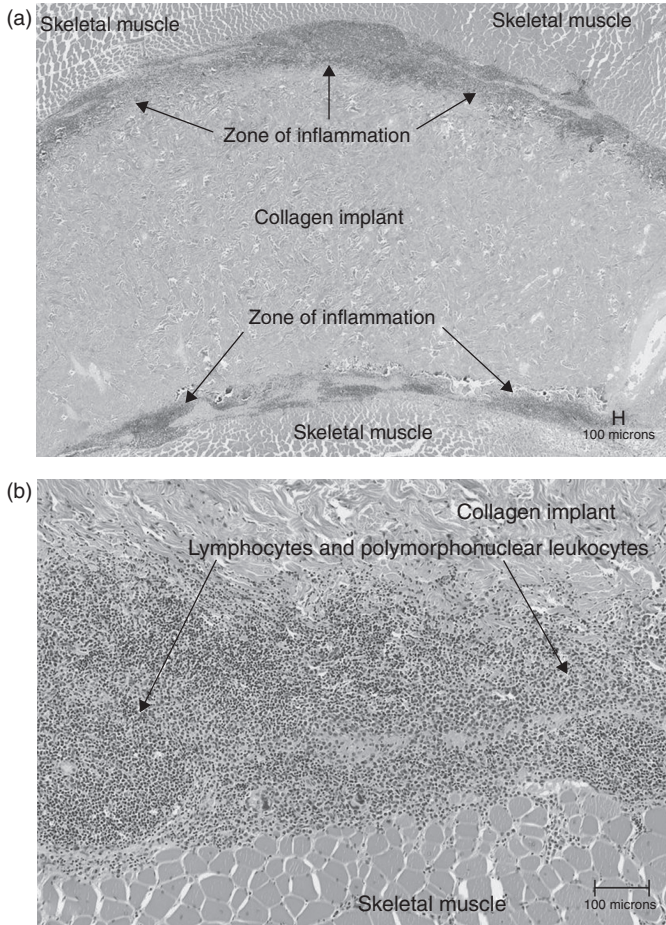
19.2.3 Morphologic assessment of systemic effects

Systemic effects are defined as those effects occurring in tissues distant from the site of contact between the body and the medical device or biomaterial. Systemic effects can be associated with leachable chemicals or degradation products released from a medical device following exposure to biological fluids and/or inflammatory cells.

Mechanisms associated with systemic effects include: chemical toxicity, non-specific cytokine activation with systemic inflammation, vasoactive effects associated with complement activation, or a specific immune-mediated response. Acute toxicity refers to adverse effects occurring within 24 h following exposure to the test article. The evaluation of acute toxicity is based primarily on clinical observations and microscopic examination of tissues is rarely performed.

Microscopic evaluation of tissues is an important endpoint in assessing sub-acute (24 h–28 days), subchronic (typically 3 months in rodents) or chronic

* Test or control article is the FDA/GLP (21 CFR 50.3) term used to designate any product implanted or administrated.



19.5 Low magnification (a) and high magnification (b) of xenogenic inflammatory response to implanted collagen. Zone of inflammation with numerous lymphocytes and polymorphonuclear leukocytes at interface between implanted collagen and skeletal muscle.

(6–12 months) systemic toxicity following repeated or continuous exposure to an intact device, device components, or extracts thereof. The rat is commonly used as a test animal for the evaluation of medical device or biomaterial systemic effects. Recommendations regarding the specific tissues to be evaluated are provided in guidance documents,^{8–12} but should include at minimum tissues representative of major organ systems and include: liver, spleen, thymus, kidney, adrenals, lymph nodes, heart, lung, and testes or ovaries. Additional tissues (e.g. brain, spinal cord, peripheral nerve, pituitary, thyroid, reproductive tract) should be included to address specific concerns relative to neurotoxicity, endocrine effects, and reproductive effects, respectively.

Microscopic evaluation of tissues for alterations indicative of systemic effects requires adequate knowledge of normal anatomy and incidental background changes in the animal being used. The interpretation of microscopic findings requires thorough correlation with clinical observations, clinical pathology (hematology and clinical chemistry) data, organ weights, and macroscopic changes noted at necropsy.

For illustrative purposes, microscopic morphologic tissue changes associated with the exposure to poloxamer-containing materials and methacrylate monomers are presented.

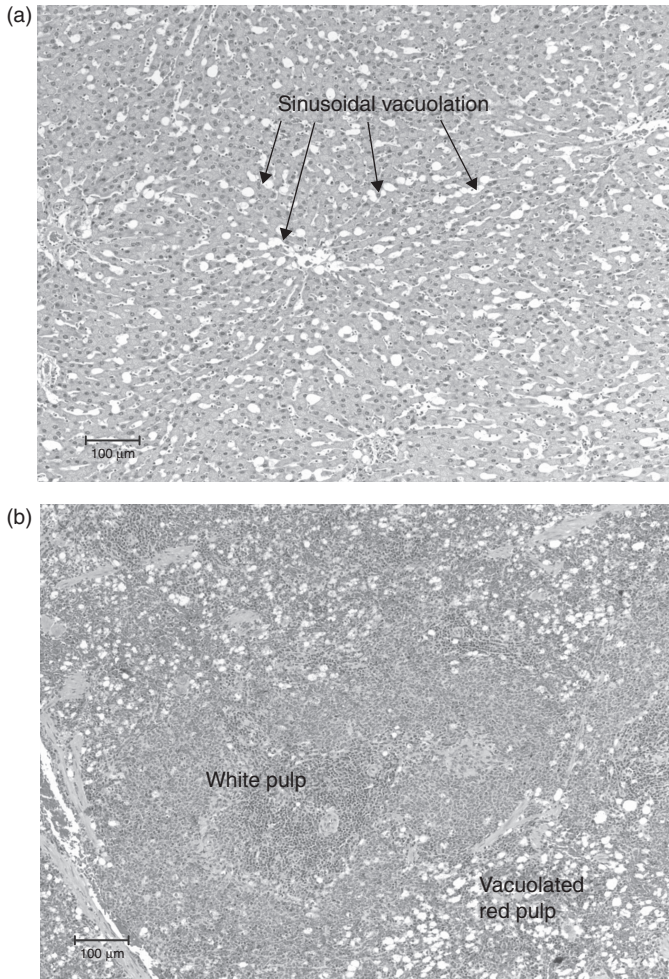
Poloxamers are synthetic block copolymers of ethylene oxide and propylene oxide with a wide range of cosmetic and medical applications.¹⁷ Poloxamers are used in drug-delivery systems and are recognized pharmaceutical excipients listed in the US and British Pharmacopoeia. There is extensive information regarding animal toxicology associated with various poloxamers.¹⁷

Systemic effects are seen following the parenteral administration of poloxamers and effects are often dose-related and limited to high dose exposure. In the case presented here, male and female Sprague-Dawley rats were implanted subcutaneously with a poloxamer-containing material. Within 7 days of implantation, some female test rats appeared pale and thin, necessitating early euthanasia (Day 8). The moribund condition of these rats was attributed to severe macrocytic anemia. Severe anemia was associated with increased splenic extramedullary hematopoiesis that was noted microscopically.

In test rats surviving to study termination (28 days), the following systemic effects were noted: decreased erythrocyte parameters in females, increased serum cholesterol levels, increased adrenal, spleen and liver weights, presence of vacuolated cells in the adrenal cortex, and vacuolated macrophages in the liver, spleen, heart and lymph nodes (Figs 19.6 and 19.7). The presence of vacuolated macrophages in multiple tissues was attributed to the systemic distribution of a soluble test article component, probably poloxamer, and/or altered lipid metabolism. Systemic exposure to specific poloxamers is associated with hypercholesterolemia and hypertriglyceridemia.¹⁷ In addition, specific poloxamers have macrophage stimulatory activity.¹⁷

This case provides an excellent example of correlation between clinical observations, clinical pathology, organ weights, and microscopic findings. It should be noted that systemic effects in this study were seen at a high dose of the test article, with a dose exaggeration factor >50× the projected human exposure.

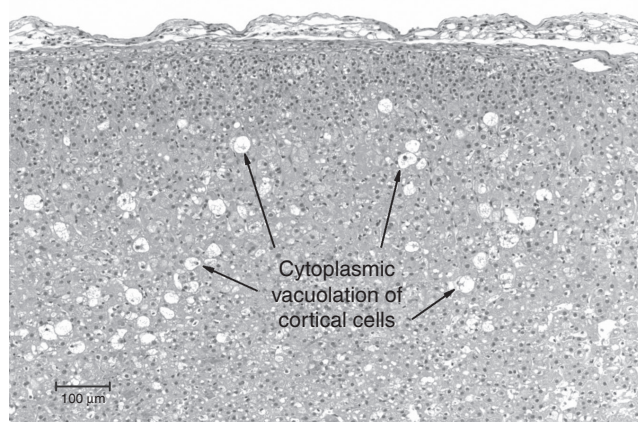
Acrylic polymers have diverse dental and medical applications. While cured polymers are stable and considered relatively inert, acrylic monomers, especially esters of methacrylate, used in the preparation of tissue



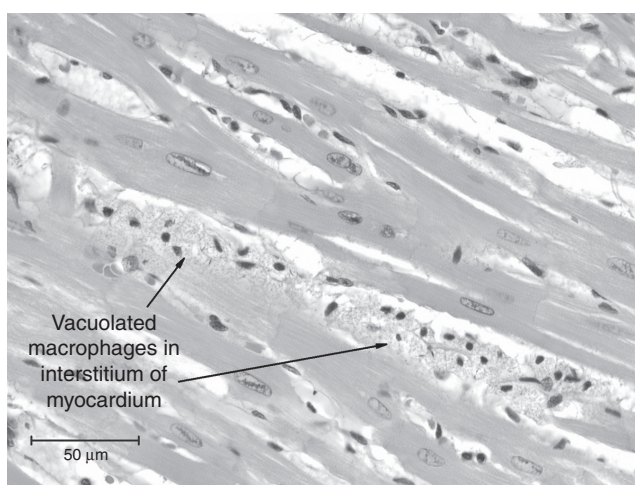
19.6 Vacuolated macrophages in (a) hepatic sinusoids and (b) splenic red pulp following subcutaneous implantation of poloxamer-containing test article.

and bone adhesives are potentially toxic. Uncured adhesives used in dental and medical applications may account for the absorption and systemic distribution of methacrylate monomers. The cardiovascular effects associated with parenteral administration of methacrylate esters are well described.¹⁸ Most methacrylate monomers cause systemic hypotension, which may be sustained or transient with subsequent rebound hypertension. Methacrylate associated hypotension is attributed to smooth-muscle relaxation and resultant vasodilation.

(a)



(b)



19.7 Vacuolated cells in adrenal cortex (a) and vacuolated macrophages in myocardium (b) following subcutaneous implantation of poloxamer-containing test article.

Hypotension is typically associated with increased respiratory rate and cardiopulmonary function returns to normal approximately 20 min following a single intravenous dose. There is little or no information available on toxicity associated with sustained parenteral exposure to methacrylate monomers.

A study was conducted to evaluate potential local and systemic effects following implantation of uncured methacrylate into the medullary cavity of the proximal tibia of five rabbits. Two rabbits receiving the uncured test methacrylate failed to recover from anesthesia and died shortly after surgery. A third test rabbit died the day following surgery during a blood

collection procedure. A fourth test rabbit was found dead on Day 2 post-surgery with foamy blood-tinged fluid around the nares and mouth.

The remaining test rabbit survived and was euthanized 4 days post-surgery. At necropsy, mottled red discoloration was present in the lungs of all test rabbits. In the two rabbits that died immediately post-surgery, the red discoloration of the lung was correlated microscopically with multifocal areas of acute congestion. Both of these animals had early degenerative vascular changes involving arterioles within and adjacent to areas of congestion (Fig. 19.8a).

Vascular changes consisted of dilation with degeneration and necrosis of medial smooth muscle. Thrombi were seen in some affected vessels. For those rabbits that died one and two days following surgery, macroscopic the red discoloration of the lung was correlated with moderate multifocal hemorrhagic necrosis. In the lungs of both animals, there was multifocal vascular necrosis and thrombosis (Fig. 19.8b). In the test animal euthanized 4 days post-surgery, microscopic pulmonary lesions were similar to those seen in animals that died acutely; however, hemorrhagic necrosis was distributed less widely.

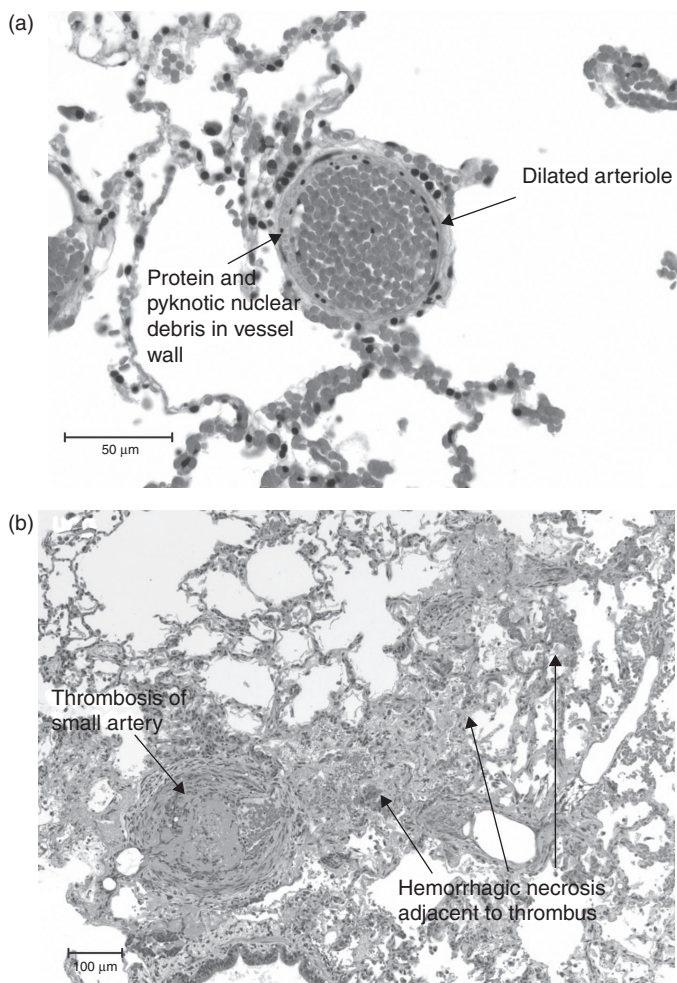
Microscopic vascular changes were well developed with endothelial degeneration or proliferation, transmural infiltration of inflammatory cells, and medial and adventitial edema and degeneration. Fibrin thrombi were present in several affected vessels. This animal also had a locally extensive area of myocardial degeneration or necrosis involving a papillary muscle in the right ventricle and a focal area of myocardial degeneration in the left ventricular subendocardium. The myocardial changes were typical of changes associated with hypoxia or endogenous catecholamine release (stress). The pulmonary changes in rabbits following sustained exposure to methacrylate monomers were attributed to prolonged pulmonary vasodilation and hypotension. While pulmonary vascular degenerative changes were probably due to prolonged vasodilation with loss of critical wall tension, vascular injury due to direct chemical toxicity must also be considered.

19.3 Assessment of the performance of biomaterials and medical devices

In this section, an overview of the importance of macroscopic assessment, imaging and submacroscopic examination, in addition to the microscopic evaluation, are discussed.

19.3.1 Macroscopic assessment

As for safety studies, evaluation of performance starts with macroscopic observations of implanted sites. Recovery of the tissues at the implant site



19.8 (a) Dilated pulmonary arteriole with acute degenerative changes in rabbit that died acutely following exposure to methacrylate monomer. (b) Pulmonary arterial thrombus and associated hemorrhagic necrosis in rabbit that died 2 days following exposure to methacrylate monomer.

can be particularly difficult when a biodegradable material is implanted. To avoid loss of the implant sites at gross evaluation, one should create or insert markers, such as pins or sutures with non-degradable threads, as anatomical landmarks at surgery, which may be supplemented by pictures. The markers should be composed of biocompatible materials so as not to interfere with any tissue reaction to the implant.

Gross findings are best reported in a purely descriptive manner. Macroscopic scoring of the implant performance, based on available

standards or scientific literature is recommended.²⁻⁶ For instance recently, the Valve Academic Research Consortium published a consensus report that described the endpoints to collect following aortic valve implantation.¹⁹

Macroscopic parameters, such as tissue integration, shape, consistency, color, visible degradation, dimensional changes of the implant, wound closure, sealing of an anastomotic junction, and anatomical implant compliance, all yield data supporting a more detailed and accurate microscopic evaluation. Some changes are not meaningful microscopically or interpretable without knowledge of the macroscopic changes (e.g. vascular graft dilation or elongation).

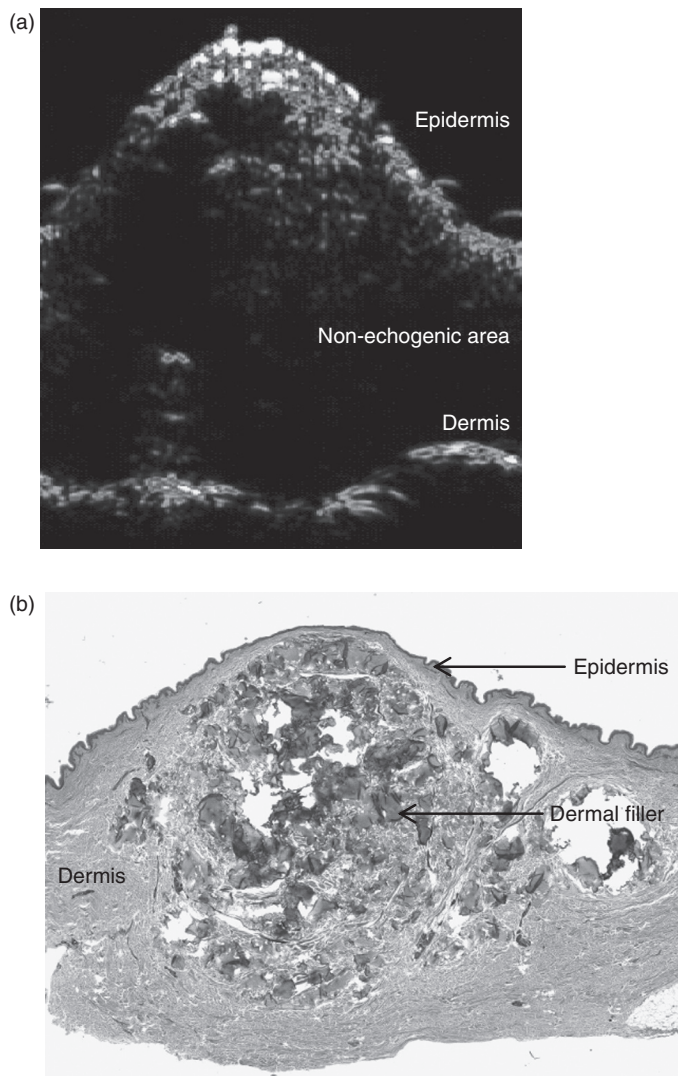
The use of a stereomicroscope or a macroscope is particularly helpful for gross evaluation, especially for small treated sites like peripheral nerves, peripheral vessels, tendons and ligaments in small animals. Scoring of the findings is advised as a way to objectively make comparisons among groups. High-resolution macrophotography (with a scale) of each site should be systematically included to assist with the microscopic evaluation.

19.3.2 Imaging sources

Data from clinical techniques such as radiography, magnetic resonance imaging (MRI), ultrasonography, computerized tomography (CT), micro-CT (μ CT), ocular imaging (e.g. optical coherence tomography), and other imaging techniques used for implanted sites are valuable in completing the macroscopic and microscopic analyses of performance. In addition, these techniques can be performed *in-life*, at regular intervals, as well as at sacrifice. In osseous sites, signs of stress shielding (redistribution of load associate with induction of osteopenia) are not observable on the histological slides, when the sampling is restricted to the implant interface. In this case, imaging results, along with the microscopic observations provide a more insightful analysis of the implant performance. Three-dimensional representations of the implanted sites, obtained with tomographic tools, enrich the interpretation of implant performance and may help to determine the most relevant plane of histological section.

In vivo fluoroscopic images focusing on endovascular implants provide an accurate evaluation of the vascular lumen narrowing in comparison to the histological images. After death there is loss of vascular tonus and muscle contraction occurs with tissue fixation. Thus, histological sections may have exaggerated vascular stenosis.

Recently, ultrasound (dermoechography) was used to analyze changes in *ex vivo* skin samples after injection of dermal fillers. Thickening of the dermis was confirmed by visualization of a dome-shaped echogenic implant (Figs. 19.9a and 19.9b). The dermoechographic findings were correlated to histological observations, indicating that the histological preparation did not induce artifactual findings or a loss of implant shape for the tested dermal fillers.²⁰



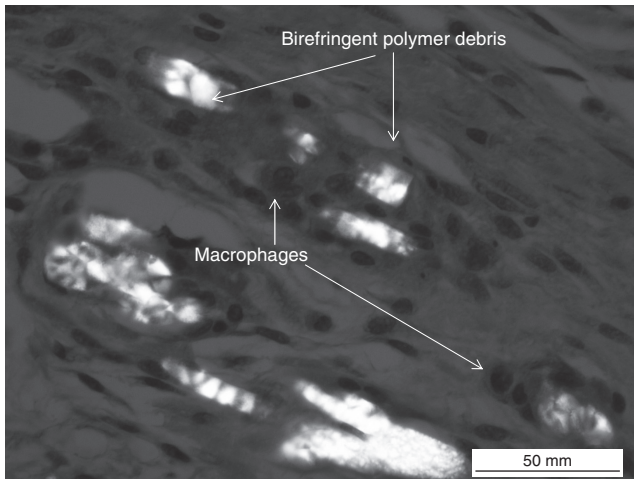
19.9 Thickening of an *ex vivo* human skin sample after injection of a dermal filler. The 20 MHz ultrasound image (a) shows two echogenic areas at the uppermost layer and at the dermis–hypodermis interface. The dome-shaped area was confirmed at histology (b) (digitized section) where the dermal filler was evidenced following staining with PAS (periodic-acid-Schiff) – alcian blue. No significant dimensional changes were observed on the histological sections compared with the ultrasound images. (Source: Courtesy of Galderma.)

19.3.3 Submacroscopy

Macroscopic examination of the histological slides before microscopic evaluation allows a topographical analysis of large sites, such as those found in spine surgery (posterolateral bone fusion, intervertebral disc replacement, spondylosyndesis [spinal fusion]), knee or hip arthroplasty, dental implants, and cardiovascular surgery. Evaluation of the frame of large tissue and implant deformation needs to be primarily addressed by a careful submacroscopic examination. The anatomic size of certain sites may prevent the microscopic observation of some material changes (e.g. signs of creep flow), detection of improper implant placement or other anatomic changes. Submacroscopy can also be correlated to histological sections and images collected by use of other techniques (e.g. CT, μ CT or Doppler ultrasound fluoroscopy investigations).

19.3.4 Microscopic assessment

A high-resolution microscope equipped with large bright field, contrast field, light reflexion, polarization, and epifluorescence instrumentation is useful for insightful observation of the implanted sites. Nanomaterials, polymer and biopolymer materials, bioceramics, composites, non-degradable and degradable metals, and alloys require adequate microscopic investigation tools for their *in situ* evaluation. For instance, light polarization will be used to detect fine material changes (deformation, stress strain) and even the intracytoplasmic presence of polymer debris (Fig. 19.10).



19.10 Fragmentation of a biotextile after 2 weeks subcutaneous implantation in a rabbit. Using polarized light, the thin polymer debris, surrounded by active macrophages, are clearly and easily detected compared with classical light microscopy. (Source: Courtesy of Johnson & Johnson MEDICAL GmbH.)

Study parameters and the scoring system are critical and should be clearly defined in the study protocol and recorded in the study report for scientific and regulatory reasons. The following are three examples that illustrate the expectations of the microscopic evaluation.

- A recent review of wound healing offers a series of parameters to assess the performance of topical treatments of wounds.²¹ Distinctive histological and immunohistochemical (IHC) parameters including re-epithelialization, epidermal differentiation (Keratin-1 IHC marker), cell migration (Keratin-6 IHC marker), proliferation (PCNA IHC marker), inflammatory response (H&E stain), as well as epidermal closure (K14 IHC basal layer marker), matrix distribution (Masson Trichrome stain), and skin remodeling with elastin fibers deposition (Van Gieson stain) were quantitatively evaluated.
- Evaluation of a drug-eluting stent (DES) in preventing restenosis might integrate updated recommendations from the international DES consensus group.^{22,23} Colonic stenting²⁴ is less frequent than DES stenting. Microscopic evaluation of the bioperformance of colonic stent could refer to the DES group work that might be adapted to enhance the histological endpoints of the colonic stenting treatment.
- Evaluation of cartilage repair could incorporate histological guidelines recommended by the International Cartilage Repair Society (ICRS)^{25,26} and/or from the Osteoarthritis Research Society International Histopathology Initiative (OARSI).^{27,28} ICRS scoring system is preferred for tissue engineered cartilage or *in vivo* repaired osteocartilaginous lesion with an implant while, for instance, drug-treated osteoarthritic cartilage is better evaluated by using the OARSI recommendations. The scoring system of ICRS II is presented in Table 19.1. Figure 19.11 shows a repaired cartilage following a 6-month implantation of a biphasic aragonite osteochondral material.

19.3.5 Pristine devices

A rigorous microscopic analysis should encompass a full non-implanted device (pristine device) including any active substances or seeded cells, as a control material for comparison with an explanted device. Thus, any subsequent biological effects on the implanted device itself will be determined based on the initial structural data obtained from the non-implanted device.

This is particularly critical evaluating the rate of implant degradation or structural changes. For example, a significant statistical difference in terms of implant area density (or implant volume) established at a given time interval must be compared with the quantitative characterization of the devices tested before implantation. This is particularly true when the implant material is porous.

Table 19.1 ICRS (International Cartilage Repair Society) II parameters*

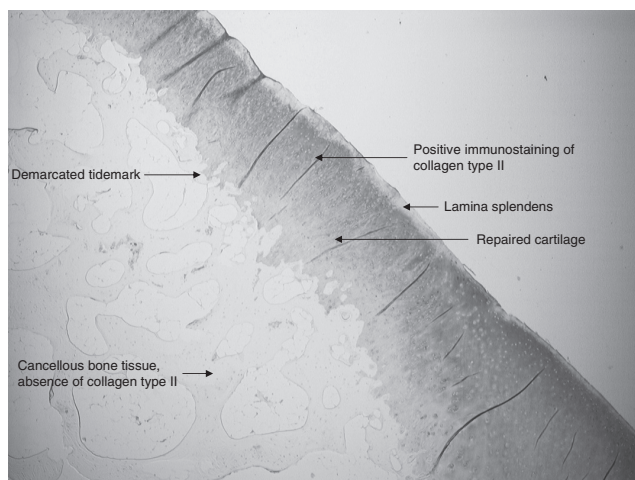
| Histological parameters | Score |
|---|---|
| 1. Tissue morphology (viewed under polarized light) | 0%: Full-thickness collagen fibers 100%: Normal cartilage birefringence |
| 2. Matrix staining (metachromasia) | 0%: No staining 100%: Full metachromasia |
| 3. Cell morphology | 0%: No round/oval cells 100%: Mostly round/oval cells |
| 4. Chondrocyte clustering (4 or more grouped cells) | 0%: Present 100%: Absent |
| 5. Surface architecture | 0%: Delamination, or major irregularity 100%: Smooth surface |
| 6. Basal integration | 0%: No integration 100%: Complete integration |
| 7. Formation of a tidemark | 0%: No calcification front 100%: Tidemark |
| 8. Subchondral bone abnormalities/ marrow fibrosis | 0%: Abnormal 100%: Normal (no infiltrates) |
| 9. Inflammation | 0%: Present 100%: Absent |
| 10. Abnormal calcification/ ossification | 0%: Present 100%: Absent |
| 11. Vascularization (within the repaired tissue) | 0%: Present 100%: Absent |
| 12. Surface/superficial assessment | 0%: Total loss or complete disruption 100%: Resembles intact articular cartilage |
| 13. Mid/deep zone assessment | 0%: Fibrous tissue 100%: Normal hyaline cartilage |
| 14. Overall assessment | 0%: Bad (fibrous tissue) 100%: Good (hyaline cartilage) |

*The criteria identified to represent the ICRS II scoring system comprise 14 parameters. Each criterion should be evaluated based on a visual analog scale²⁶ and graded from 0 to 100. The best and the worst features are described for each parameter.

Structural characteristics of the non-implanted devices are used to describe the *in vivo* behavior of the implanted materials. Signs of material changes such as erosion, swelling, creep flow, cracks, fragmentation, delamination, or break-down will invariably impact the host tissue response. Additionally, unexpected local tissue reactions could be explained by material changes only detectable by changes in the histological stain affinity (pH changes) of the implant material observed for some biodegradable polymers (polylactide acid, lactide caprolactone copolymer, or other biodegradable polymers). Therefore, the description of material changes should be included as part of the tissue response analysis.

19.3.6 Control sites

Control materials are implanted in the same manner as test materials. Animal factors (e.g. age, strain, and gender) and local tissue constitution



19.11 Full repair of a goat osteochondral defect (6×10 mm), 6 months following implantation of Agili-C™, an acellular biphasic aragonite-hyaluronate implant. Immunodetection of collagen type II, a specific hyaline cartilage marker. The composite material was fully resorbed and replaced by a newly formed cancellous bone tissue. The sham-operated control site was not repaired. (Source: Courtesy of Cartiheal Ltd, Israel.)

(e.g. level of vascularization, architecture and local biomechanical stress) must be standardized before any comparison of implant performance.

Analysis of sham-operated sites provides a baseline for healing or effects of surgical trauma in the animal model or reparative system and facilitates interpretation of implant success or failure following normalization of the healing data. In regenerative medicine, the determination of the *restitutio ad integrum* (full healing with complete recovery to the original tissue) of implant-treated tissues also requires intact and undisturbed samples of tissue as naïve controls.

19.4 Processing and sectioning of specimens

Depending on size, specimens (tissues containing implants) should be totally submerged in the fixative at a ratio of 1 part specimen to 10–20 parts fixative for adequate fixation. The rate of tissue penetration of the most commonly used fixative (i.e. 10% neutral buffered formalin solution [NBF]) is 2 mm per day.

The method for specimen preparation is determined by considering the physical and chemical stability of the implant through the histological processing steps. That is why, before preparation, the implant alone should be first tested through all the histological treatment steps, to evaluate any material change (swelling, material dispersion, dimensional changes, material loss, see Section 19.3.5). This step may prevent misinterpretation of the

local tissue effects and implant reaction. For example, alginates and a few chitosan materials dissolve in 10% NBF. To overcome this situation, other fixatives such as Carnoy's liquid or alcohol-formalin (90:10) may be found to have minimal effects on such a specific material.

In addition, the endpoints of the study will determine method of specimen preparation.

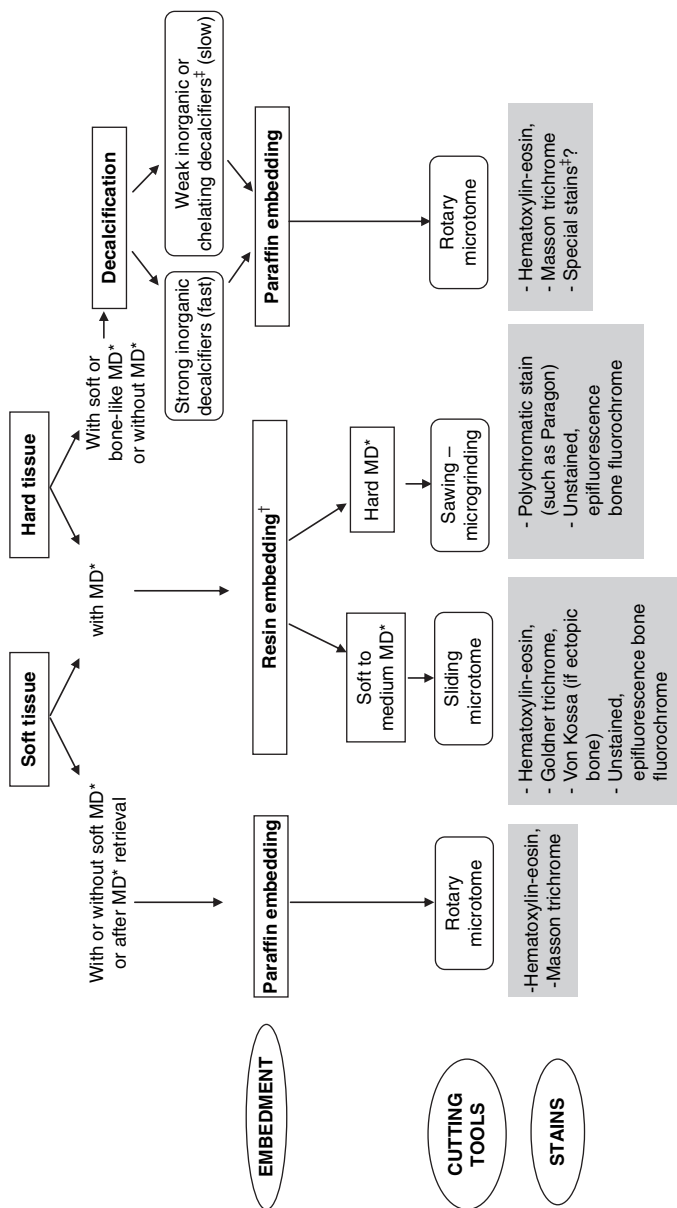
Examination of the endothelialization of a vascular medical device by using a scanning electron microscope is best performed following vascular perfusion fixation of the animal or implanted sites. Perfusion fixation is also recommended for the study of implanted mandibles and treated teeth. Other fixatives may be used for optimal organ preservation, for example, testes are far better fixed and interpretable in Davidson's fixative than in routine 10% NBF. Bone tissue is known to be well fixed in ethanol solution but inflammatory and interfacial pathological changes are best investigated with the use of 10% NBF. The study of the glycosaminoglycans in hyaline cartilage tissue will be preferably conducted following fixation in 10% phosphate-buffered formalin with 1% cetylpyridinium chloride as demonstrated by Engfeldt.²⁹ Cetylpyridinium chloride prevents the glycosaminoglycans from leaching as observed with routine 10% NBF.

The IHC determination of biomarkers requires specific conditions of preparation such as limited time of tissue fixation (<48 h) before specimen embedding, otherwise the IHC results are potentially compromised.

Embedding in paraffin is used for most soft tissues (organs, subcutaneous tissue, muscle, tendon, ligament, cartilage, meniscus) while hard tissues (bone, dental tissues) are mostly embedded in polymethylmethacrylates (PMMA) resins.^{30,31} However, the physical nature of the implant within the tissues can lead to different technical choices. For example, resin embedding is recommended for soft tissues implanted with a hard implant.

Methacrylate-based materials such as bone cement, intraocular lenses, polylactic acid polymers, and other polymer materials may dissolve or deform in methylmethacrylate, the monomer of the polymethylmethacrylates (PMMA) resin embedding medium. To solve this issue, other plastic embedding media such as epoxy or glycol methacrylate (GMA)-based resins can be used as alternatives.

When bone tissue contains a soft implant or a bone-like material, or in the case of osteochondral specimens, paraffin-embedding might be a quick and cheap solution for subsequent histological evaluation, but first requires an appropriate decalcification process. As a consequence however, old and newly formed bone cannot be distinguished. In addition, the implant might disappear during processing and any evaluation of calcium-based bone labeling then becomes impossible. Figure 19.12 depicts potential technical pathways for the treatment of specimens according to the type of the



19.12 Suggested scheme for routine selection of embedding media, cutting process and stains. *MD: medical device. †PMMA, GMA, SPURR: these hydrophobic or hydrophilic resins are selected based on their compatibility with the MD. ‡Special stains mostly successful after soft decalcification.

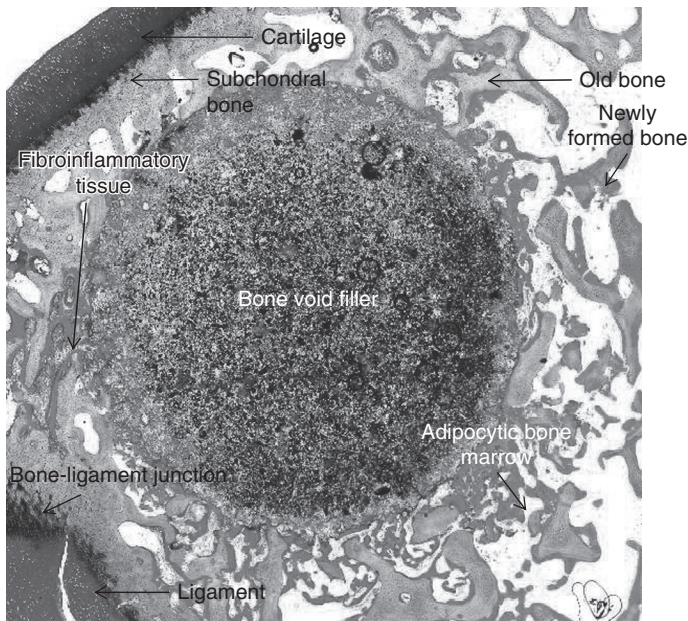
tissues and implants. Commonly used cutting techniques are also shown in Fig. 19.12.

19.5 Staining recommendations

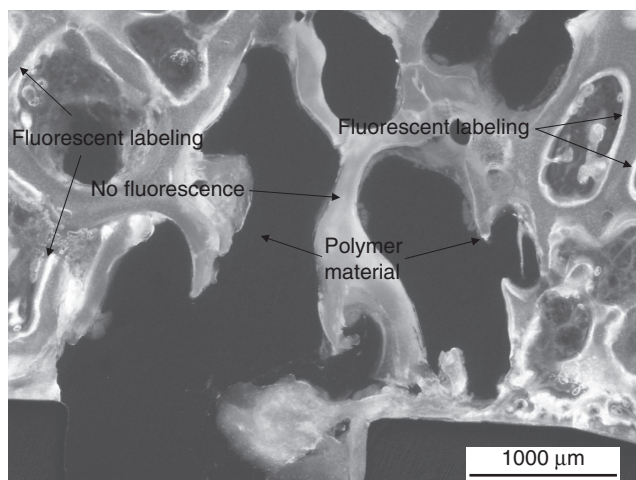
Among the hundreds of staining techniques available, a few are routinely employed for the assessment of implant–tissue interface. Many reviews present the available staining techniques.^{30–34}

For soft tissues analysis, the ideal general stain should make it possible to identify clearly cellular populations including inflammatory cells, pre-existing and newly formed extracellular matrix, parenchymal tissue, and the implant material. For hard tissues, the ideal general stain should enable one to clearly identify the soft tissues (fibroconnective tissue, bone marrow, mucosal tissue, gingival structure, tendon, and ligament), the cellular population including inflammatory cells, old bone and newly formed bone, cement lines, osteoid rims, meniscal and cartilage structures, dentinal tissue, and implant material (Fig. 19.13).

Figure 19.12 depicts the choice of stains according to the type of tissue and processing as followed in our laboratory.



19.13 Digitized section. Full osteointegration of a bone void filler after 4 weeks' implantation in the distal femur of a rabbit. The modified Paragon staining of this non-decalcified ground section permits one to discriminate between old bone (light gray) and newly formed bone (dark gray), fibroinflammatory tissue, cartilage, subchondral bone, ligament and junctional areas (in metachromatic color).



19.14 Bone fluorescent labeling (yellow-gold linear fluorescence: white lines in the picture). Visualization under ultraviolet-light of a tetracycline double labeling in sheep (double injection of tetracycline at 5 day intervals). Observation made after 4 weeks' implantation and diffusion of a polymer material within the cancellous bone. The bone tissue embedded into the polymer material does not show fluorescent lines of mineralization while the bone tissue adjacent to the polymer material appears as living bone with marked signs of mineralization. With routine stain (modified Paragon stain), this difference was not visible and the bone frame appeared similar within the polymer and at the periphery of the polymer material.

Staining of unimplanted material that is processed, embedded, and stained under the same conditions as for the treated sites, will serve as baseline control (see Section 19.3.5).

Special staining techniques can be used for the detection of specific tissues or cells or implant components, or activity in the tissues or cells surrounding the implant (Fig. 19.14). These special staining techniques include histochemistry, enzyme histochemistry, immunohistochemistry, and molecular histology. Table 19.2 presents a few examples of staining techniques routinely used in our laboratory.

19.6 Qualitative and quantitative pathology used in the evaluation of biomaterials and medical devices

Qualitative examination of implant sites is a first step aimed at selecting properties that should be measured, identifying inappropriate sites to be excluded (e.g. implant mispositioning, signs of contamination, surgical trauma), adjusting the areas of investigation, standardizing regions of

Table 19.2 Examples of staining techniques and endpoints for tissues containing implant

| Technique | Stain | Endpoints |
|----------------------------|---|---|
| <i>General staining</i> | | |
| Bichromic stain | Hematoxylin-eosin | Analysis of pathological changes at cellular level |
| Trichromic stain | Masson's trichrome | Study of extracellular matrix, fibroplasia, fibrosis |
| | Goldner's trichrome | Soft and hard tissues stain on microtomed plastic sections |
| Pentachrome stain | Movat's pentachrome | Soft and hard tissues stain on microtomed plastic sections |
| Polychromatic stain | Modified Paragon stain* | Soft and undecalcified hard tissues stain on ground plastic sections |
| <i>Special staining</i> | | |
| Histochemistry | Safranin-O | Detection of glycosaminoglycan formation in cartilage tissue |
| | Fast Green | |
| | Von Kossa | Detection of mineral deposits (ectopic bone, dystrophic mineralization) |
| | AgNor | Detection of osteopontin |
| Histoenzymology | Periodic-acid-Schiff | Basement membrane, glycogen |
| | Verhoeff or Van Gieson | Detection of elastin lamina |
| | TRAP (tartrate resistant acid phosphatase) | Detection of osteoclasts (bone resorption, osteoporosis treatment) |
| | Alkaline phosphatase | Detection of osteoprogenitor cells, osteoblasts, odontoblasts |
| Immunohistochemistry (IHC) | Immunodetection of antigen X and biochemicals | Detection of biomarkers (inflammatory cells, neovascularization, neurofilaments (in nerve regeneration), extracellular components like collagen type I for connective tissue and bone, type II for cartilage tissue, type III for organs and early stage repair |
| Molecular histology | <i>In situ</i> RT PCR Hybridization <i>in situ</i> | Early stage of synthesis detection, study of inflammation (cytokines), other gene expression |
| Epifluorescence | Fluorochrome bone labeling (performed <i>in vivo</i> , without decalcification) | Determination of the bone mineralization rate, remodeling, signs of toxic effects of implants on bone |
| Polarization | No stain, or use of a specific stain such as Red Picosirius | Detection of polymers and crystalline materials Collagen orientation and maturation |

*Modified Paragon stain.

interest (ROI), and defining the features that may usefully be compared statistically.

At least $N = 10$ sites randomly selected per group should be included in the studies for robust results. $N = 10$ sites per group is also recommended by standard ISO 10993-6 for histological semi-quantitative analysis (Fig. 19.15). Lower numbers include increased α and β risks during statistical analysis. In addition, biological versus statistical significance must always be considered in the interpretation of the results.

| Group | Test | | | | | | | | | | Control | | | | | | | | | |
|--------------------------------|------|--|--|--|--|--|--|--|--|--|---------|--|--|--|--|--|--|--|--|--|
| Animal/site | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| PMNs | | | | | | | | | | | | | | | | | | | | |
| Lymphocytes | | | | | | | | | | | | | | | | | | | | |
| Plasma cells | | | | | | | | | | | | | | | | | | | | |
| Macrophages | | | | | | | | | | | | | | | | | | | | |
| Giant cells | | | | | | | | | | | | | | | | | | | | |
| Necrosis | | | | | | | | | | | | | | | | | | | | |
| SUB TOTAL (X2) | | | | | | | | | | | | | | | | | | | | |
| Neovascularization | | | | | | | | | | | | | | | | | | | | |
| Fibrosis | | | | | | | | | | | | | | | | | | | | |
| Fatty infiltrate | | | | | | | | | | | | | | | | | | | | |
| SUB TOTAL | | | | | | | | | | | | | | | | | | | | |
| TOTAL | | | | | | | | | | | | | | | | | | | | |
| GROUP TOTAL (irritation score) | | | | | | | | | | | | | | | | | | | | |
| Traumatic necrosis | | | | | | | | | | | | | | | | | | | | |
| Hemorrhage | | | | | | | | | | | | | | | | | | | | |
| Fibrin | | | | | | | | | | | | | | | | | | | | |
| Foreign debris | | | | | | | | | | | | | | | | | | | | |
| Material degradation | | | | | | | | | | | | | | | | | | | | |

19.15 Semi-quantitative histopathological analysis of local tissue effect

ISO 10993-6. Semi-quantitative histological evaluation of local tissue effects of each implanted site is graded with a scoring system ranging between 0 (none) and 4 (severe). The irritation score of the test and control group is calculated from the histological semi-quantitative analysis. The IRS reflecting the inflammatory intensity and the local tolerance reaction is determined by subtracting the irritation score of the control group from the score of the test group. A negative difference will be recorded as zero. The IRS is graded as non-irritant (0.0–2.9), slightly irritant (3.0–8.9), moderately irritant (9.0–15.0), or severely irritant (>15.0). Here, the ISO table is implemented with the calculation of the group mean and standard deviation that allows accurate comparison of the inflammatory types and heterogeneity of the tissue responses. (SD in the figure denotes standard deviation.)

The irritation score of the test and control groups is calculated from the histological semi-quantitative analysis.

The Irritant Ranking Score (IRS) reflecting the inflammatory intensity and the local tolerance reaction is determined by subtracting the irritation score of the control group from the score of the test group. A negative difference will be recorded as zero. The IRS is graded as non-irritant (0.0–2.9), slightly irritant (3.0–8.9), moderately irritant (9.0–15.0), or severely irritant (> 15.0). Here, the ISO table is implemented with the calculation of the group mean and standard deviation that allows accurate comparison of the inflammatory types and heterogeneity of the tissue responses.

Quantitative histomorphometric evaluation is used for safety issues such as the measurement of inflammatory cellularity following marking of specific inflammatory cells or evaluation of local cytokine expression.

Computer-assisted quantitative histomorphometry, rather than a qualitative or semi-quantitative analysis, is a good way to evaluate and compare materials, reactions, and reconstitution of tissues or organs, whenever performance evaluation is conducted.

This method permits measurement of morphologic characteristics of tissue reactions such as infiltrating cells, extracellular material and components, as well as the implant material itself. Measurements are based on surface area, length, circumference, angle, number of objects, optical density distribution, or any other quantitative parameter. In practice, by using a computer system for images analysis, the measurements are carried out in a determined and standardized ROI. In order to be representative of the tissue and implant reaction, ROIs should be carefully selected and accurately defined. For example, the bone area density measured around an eluting drug implant could provide totally different results when the measurements are performed either within a 250 or 1000 μm width around the implant. What is statistically different between two groups of treatments when the ROI is 250 μm width, could be lost with a larger ROI set at 1000 μm width. Thus, the most relevant quantitative expression of the performance of an implant may depend on the definition of the ROI set for quantitative characterization.

Computer-assisted quantitative histomorphometry provides accurate and objective metrics allowing statistical comparison between implant treatments. In dental, bone (cranio-maxillofacial, vertebral, long, cortical, trabecular, or ectopic) and vascular healing, quantitative approaches by histomorphometry are commonly used.^{35–37} Fine comparison and powerful discrimination between test and control implants are established when close peri-implant tissues and implant measurements are carried out. The time course of healing or material degradation can be quantitatively characterized by this method.

For evaluation of bone implants or bone substitutes, a commonly used parameter is the bone-to-implant contact percent (BIC %), which reflects the level of osteointegration and osteoconduction at the surface of implants. In addition, bone area density is a useful parameter that often correlates well with material degradation (osteotransduction), thus allowing qualifying an implant as a bone substitute.

In vascular treatment, a sensitive parameter is thickening of neointimal tissue. Measurement of neointimal hyperplasia indicates the effect and performance of an endovascular implant on the prevention of vascular re-stenosis.

In general, computational histomorphometric approaches are less often applied in soft tissue repair than in hard tissues. However, we recently developed an approach for the quantitative evaluation of soft tissue repair by measuring the speed of implant integration into soft tissues (manuscript in preparation). Using two specific staining methods (picrosirius red for collagen determination and Feulgen and Rossenbeck for DNA staining), the extracellular matrix (ECM) and the cellularity (C) were respectively measured within abdominal reinforcement meshes. The rate of tissue ingrowth (RTI %) was established based on an integrative formula, considering together the amount of extracellular matrix and the amount of cells within the mesh.

When the integrative formula is divided by the healing time (ht) of the study, the result provides the speed of tissue ingrowth (STI). The STI is presented as being an equation with $STI = RTI\% \cdot ht^{-1} = ECM\% \cdot ht^{-1} + C\% \cdot ht^{-1}$. To make it simpler, the variable ht might not be used, then the rate of tissue ingrowth $RTI\% = ECM\% + C\%$.

This method was used to demonstrate statistically significant delayed tissue ingrowth in diabetic versus healthy rats. To our knowledge, this is the first quantitative demonstration of impaired healing in diabetic rats. This approach is also applicable to soft tissue regeneration, wound healing, soft tissues augmentation, tendon and ligament repair evaluation, etc.

19.7 Ultrastructural pathology

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are the most powerful methods for evaluating the ultrastructure, architecture, and pathological changes at the cellular and subcellular level with measurements in the micrometer to nanometer range.

19.7.1 SEM

The main use of SEM is to obtain topographic images with a magnification range of 10–10 000 \times to describe biological, organic, and inorganic materials. The three-dimensional images obtained supplement light microscopy analysis limited to two-dimensions. These techniques are used with

3D-material structures (e.g. porous, reticulated or thread-like defects on the surface of materials such as orthopedic implants and packaging materials), blood-flow orientation and maturation of endothelial cells growing onto a vascular graft (e.g. cellular confluence and mesothelialization patterns upon peritoneal medical devices) (Fig. 19.16). Surface and coating defects (e.g. corrosion, pitting, delamination, cracks), crystallographic and grain changes are well characterized using SEM. Retrieved intraocular lenses are analyzed by SEM to evaluate surface material defects and the extent of protein deposition as discussed in Section 19.8. The initial surface characteristics of critical devices used in the cardiovascular system or in neurosurgery are perfectly described by SEM (e.g. roughness, alterations, cracks, fissuration, deposits, foreign particles). Suspicion of device infection or biofilm development is easily documented by SEM (Fig. 19.17). These methods are also recommended for characterization of blood/device characterization, as recommended by the ISO 10993-Part 4 standard. Inferior vena cava filters, vascular prostheses, or endoluminal stents can be fully characterized following animal implantation. Implants (vascular prostheses, ocular implants) retrieved from human patients are regularly evaluated by SEM to determine their mode of failure.

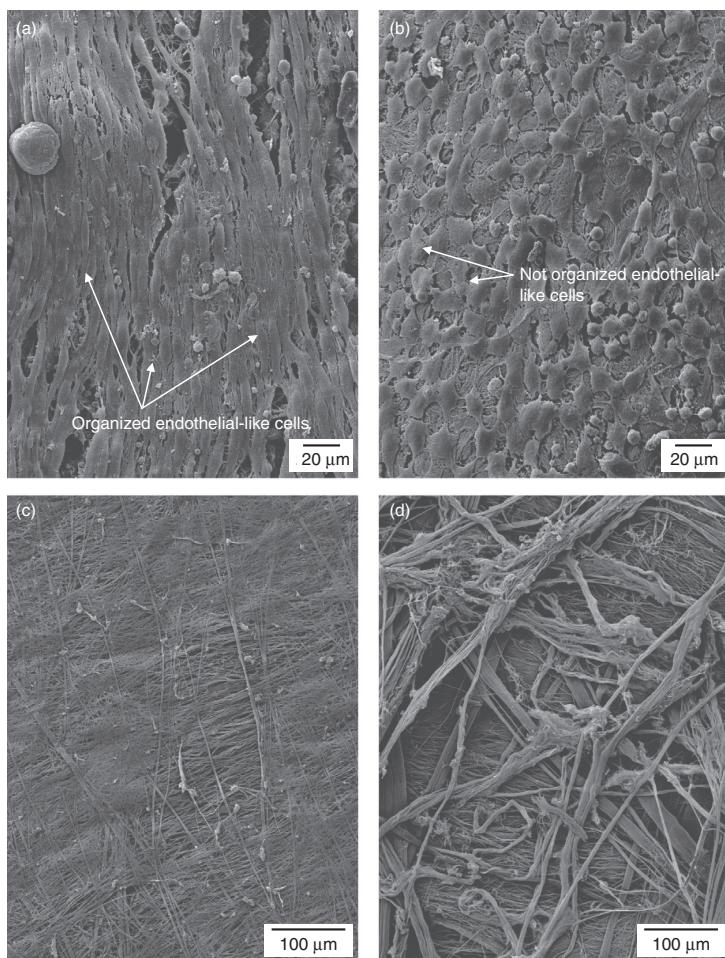
Due to the electron beam bombardment used to visualize specimens, fragile structures might be deformed even dynamically during the observation. Conventional methods of preparation of biological specimens (fixation, desiccation, high vacuum) could induce artifacts and this may complicate interpretation of the images. The use of a control (unimplanted material, normal tissues) and calibration (scale) of the SEM pictures will help fine-tune the interpretations.

Low beam energy and environmental SEM are tools that permit observation of wet specimens or beam-sensitive material such as small free components, particles, granules of material, polymeric and fragile biological structures.^{38,39}

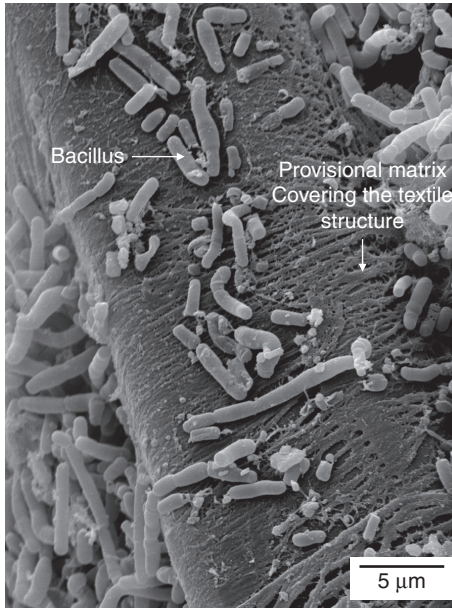
Composition and elemental identification of the material (metals, alloys, composites and ceramics based-material) can be obtained by using SEM-X-ray microanalysis with a 10–100 ppm limit of detection.^{38,39} Detection and characterization of released micro and nanoparticles derived from the implanted device are possible within the surrounding tissues and limits of detection. Contamination by foreign bodies/particles or elements can also be easily documented by SEM-X-ray analyses.

19.7.2 TEM

Subcellular injury, extracellular macromolecular changes, and contamination with micro and nanoparticles are not usually clearly manifest under



19.16 Percutaneous aortic valve in a heterotopic sheep model. Neoendothelium observed by SEM on the inflow and outflow sides of the leaflets after 4 weeks of implantation. A marked endothelialization with confluent and organized cells was observed on the outflow side of the leaflet (a), while on the inflow side (b), a delayed growth of endothelial-like cells (no confluence, no organization) can be seen. Before implantation, the outflow side of the leaflet material appeared as a smooth surface (c), while the inflow side exhibited a rough surface (d). Typically, it is hard to observe such a pattern of neoendothelialization using histology methods. (Source: Courtesy of nvt GmbH.)

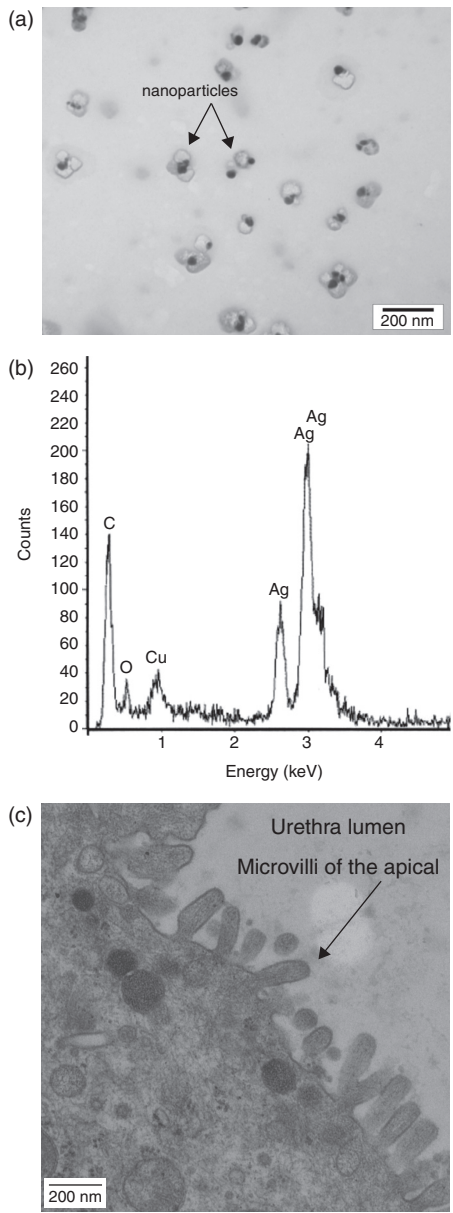


19.17 SEM image of bacterial proliferation at the external side of a human vascular prosthesis. The explant was received not fixed. The bacteria might have mostly proliferated after sampling.

light microscopy due to the limits of resolution. TEM analysis clarifies these biological events and reveals ultrastructural changes and foreign body contaminants. Interpretation of small TEM biopsies (≤ 2 mm) is guided by histological observations.⁴⁰

High-quality, ultrastructural images depend on the method of processing used, which requires consistency in fixation, post-fixation, epoxy embedding, and ultramicrotome sectioning at 70 nm thickness or less. As size, morphologic structure, and location are the three main criteria governing interpretation of ultrastructural images,⁴⁰ the TEM should be calibrated before analysis.

Biopsy of healthy tissue prepared under the same conditions as a biopsy of treated tissue must be used as control to observe ultrastructural pathological changes.⁴⁰ Whenever compatible with the study design, a sample of the unimplanted medical device (test or control) should be examined to understand the control. This is particularly necessary when nanomaterials are evaluated. By recognizing the control nanostructure, normal intracellular inclusions, normal organelles, and electrode artifacts, misinterpretation can be avoided. Classical preparation of specimens for TEM can destroy fragile nanostructures and cryo-ultramicrotomy may be an alternative technique of preparation, preserving those fragile nanostructures as shown in Fig. 19.18. In these last illustrations, a transmission electron microscope



19.18 TEM analysis of a urethral catheter coating following cryo-ultramicrotomy to preserve the coating (a). The silver nanoparticles of 30 nm were chemically characterized by the mean of EDS (b). The peak of copper (Cu) is artefactual and relates to the copper grid supporting the ultrathin section. No silver particles were found at 4 days following intermittent insertion of the coated catheter in the rabbit urethra. (c) TEM section of urothelial cell devoid of silver nanoparticle.

equipped with a solid state energy dispersive spectrometer (EDS) was used to identify the silver nanoparticles dispersed within the implant coating. The EDS method is particularly appropriate for ultrathin sections. After electron bombardment of the ultrathin section, the resulting X-rays have characteristic energy and wavelength of each element detected by the EDS spectrometer.

Ultrastructural analysis of the biological structure and morphology of the cells and tissues (cell swelling, lesions of organelles, signs of degeneration, disordered pattern of the extracellular matrix) provides clues about the etiologic agent. For example, some toxic products induce lesions that are highly specific for certain tissues, cells, or organelles.⁴⁰ Titanium dioxide particles, mainly accumulated in liver and spleen, could be retained for over 30 days in these tissues in mice or rats.⁴¹ Recently, the direct effects of iron oxide nanoparticles on human aortic endothelial cells were investigated, showing that cytoplasmic vacuolation, mitochondrial swelling, and cell death were induced in human aortic endothelial cells *in vitro*.⁴² The location of the agent (extracellular, intracytoplasmic, intranuclear) is important. Recently, cellular internalization of zinc oxide nanoparticles (used in cosmetics and sunscreens) that was associated with signs of cytotoxicity and genotoxicity in primary human epidermal keratinocytes was demonstrated by TEM.⁴³ We have recently observed intracytoplasmic and intranuclear nanoparticles in hepatocytes following intravenous injection of the nanomaterial in rats.

In the field of nanoparticle distribution, storage, and excretion, TEM is a highly useful tool, allowing analysis of the nanoparticles, as well as cellular and subcellular changes induced by their uptake.

19.8 Morphologic assessment of ocular medical devices

Ocular medical devices include those that come in contact with the exterior of the eye (e.g. contact lenses and nasal canalicular plugs) and those that are implanted within structures of the globe (e.g. subconjunctival implants, scleral plugs and implants, suprachoroidal implants, intraocular lenses, and intravitreal implants).⁴⁴ In general, ocular medical devices are tested for evidence of biocompatibility (i.e. absence of localized irritation or toxicity), but if the ocular device elutes a drug, then additional organs and tissues in the body are often evaluated for systemic toxicity. For ocular devices, the most commonly used laboratory animal for testing is the albino rabbit, followed by the dog, minipig, and monkey. Regardless of the animal used, the examiner needs to be aware of the general responses of ocular structures to physical or chemical injury. The examiner must also be familiar with the ocular structures and spontaneous background findings that are uniquely

associated with a particular species. This is important in order to determine if microscopic ocular findings are of toxicological importance.

19.8.1 Rabbit model

New Zealand White rabbits are used because their eyes are large enough to accommodate medical devices, such as contact lenses or intraocular implants, and their albino condition allows for easy detection of ocular irritation. This strain of rabbit has been used for years in the evaluation of clinical ocular irritation with the use of the Draize scoring system and more recently with the slit-lamp biomicroscope and the McDonald-Shadduck scoring system.^{45,46}

Since the human eye is pigmented, occasionally pigmented rabbits (e.g. Dutch-Belted and New Zealand Red) are used for testing a particular ocular medical device, especially if light absorption or melanin-binding are issues.

There are several features of the eye of a rabbit that are unique.^{47,48} Funduscopically, rabbits have a superiorly located optic disc with retinal blood vessels extending in nasal and temporal directions along a horizontal plane (i.e. merangiotic retinal vascular pattern). Myelinated axons of retinal ganglion cells are also located in this plane (i.e. medullary rays). Ventral to medullary rays is a specialized area of the retina, the area centralis or visual streak. This elongated area contains an increased number of cone photoreceptors. Anteriorly, ciliary processes (i.e. iridal processes) attach to the posterior aspect of the iris.

In addition to the globe, rabbits have several orbital glands. A small lacrimal gland is located in the superior, temporal and anterior aspect of the orbit and a larger accessory lacrimal gland is located in an anterior and ventral position in the orbit.⁴⁹ Microscopically, lacrimal glands frequently have a few aggregates of mononuclear inflammatory cells and occasionally areas of focal glandular atrophy. Additional orbital glands include the Harder's gland, a large gland located in the nasal aspect of the orbit and a gland associated with the third eyelid (membrane nictitans). The Harder's gland is composed of a white lobe and a pink lobe. Microscopically, the white lobe has smaller lumina and stains more intensely, whereas the pink lobe has larger lumina and contains larger lipid droplets. Common microscopic findings include focal atrophy and mononuclear cell infiltration. Mononuclear inflammatory cell infiltration may occur as a spontaneous finding in the episclera adjacent to the corneoscleral junction (limbus) and heterophils are commonly observed in the conjunctiva.

In general, there are several spontaneous non-infectious and infectious ocular conditions that occur in rabbits. Non-infectious conditions include

pseudopterygium, a condition that has been reported in several breeds of rabbits and consists of a flap of conjunctiva extending over the cornea.⁵⁰ Corneal dystrophy may occur in Dutch-Belted rabbits and is characterized by thin and disorganized corneal epithelium, thickened epithelial basement membrane, irregular basement membrane–stroma junction, and disorganization of the subepithelial corneal stroma.⁵¹ Enlargement of the globe (buphthalmos) with diffuse corneal edema occurs sporadically in New Zealand White rabbits as an inherited condition causing glaucoma.⁴⁷ Spontaneous hypertrophy of retinal pigment epithelial cells of rabbits occurs sporadically in several breeds. The hypertrophied cells contain an accumulation of lysosomes and lipofuscin and eventually become large, rounded, and detached with extension into the subretinal space. This finding occurs commonly around the optic disc and appears to be more extensive in Dutch-Belted rabbits with more of a diffuse involvement. Focal retinal detachment might occur with time. Infectious ocular conditions are infrequent in laboratory rabbits, but subclinical infections of *Encephalitozoon cuniculi* have been associated with rupture of the lens capsule and phacoclastic lens-induced inflammation.⁵²

19.8.2 Canine model

The beagle dog may be used for testing external ocular medical devices, such as intracanalicular (punctal) plugs or certain types of intraocular devices. Dogs are easy to handle, which is helpful in clinical examinations, and the canine eye has many similarities to the human eye. Human and subhuman primate eyes have a macula and the canine eye has an *area centralis*, which has similarities to the macula. The canine eye also contains a specialized area of the choroid, the *tapetum lucidum*, which is missing in the human eye. It is located just beneath non-pigmented retinal pigment epithelium and the choriocapillaris. In the dog, the structure is composed of epithelial cells (*tapetum cellulosum*) with uniform, electron-dense, zinc-containing rods. The rods are arranged in a uniform pattern to reflect light. Funduscopically, the *tapetum cellulosum* is hyperreflective when the overlying sensory retina is thinned (usually the outer retina) and may be a target of toxicity from such agents as beta-adrenergic blockers.⁵³

19.8.3 Porcine model

The minipig has eyes that are large enough to be used with ocular medical devices and has morphologic features that are similar to those of the human eye.⁵⁴ Depending on the ocular medical device, the minipig may be more difficult to handle for ophthalmic examinations than a rabbit or dog.

19.8.4 Non-human primate model

Non-human primates have eyes that are the most similar to human eyes, but due to their expense and their special maintenance requirements, they are generally used only when there is an essential need for similarity in the ocular anatomy of the test animal with humans. Monkeys may have spontaneous background findings. For example, cynomolgus monkeys often have an infiltration of mononuclear cells in the ciliary body or choroid that are considered non-specific and not associated with the test device.⁵⁵ Cynomolgus and rhesus monkeys may have a loss of macular ganglion cells resulting in temporal atrophy of the optic nerve.⁵⁶ This idiopathic finding is best observed by examining axons in a cross section of the retrobulbar optic nerve.

19.8.5 Histological preparation and evaluation

An accurate microscopic assessment of ocular irritation or localized toxicity from a medical device requires good histological sections. A good histological section for microscopic examination is one that is in the correct plane and has few artifacts. A good plane of section is one that is in the area of the device. For example, a contact lens covers the entire cornea, so a central sagittal section along a vertical plane through the center of the cornea, the pupil, and the optic disc would be preferred. Histological sections of all globes should be along the same plane for accurate comparisons within the study and between similar studies. Some ocular medical devices are implanted within the globe. If the device is degradable and not located along this standard central plane, then the plane of section may be moved from the vertical position. If the device is located near the outer aspect of the eye, then the standard section may be obtained followed by an additional section through the device. If the device is releasing a substance, then step sections at a uniform distance may be necessary to examine the portion of the globe exposed to the released substance. If the intraocular medical device is hard, then instead of using a wax, such as paraffin, as the embedding medium, the globe is embedded in methacrylate plastic. Sections are prepared by sawing and grinding the blocks using the Exakt™ system or equivalent (see Section 19.4 and Fig. 19.12).

Microscopic evaluation of an ocular medical device starts at the time of enucleation.^{44,57} To minimize postmortem artifacts, enucleation should occur as soon as possible after the animal is euthanized. The globes should be gently removed and cleaned of extraocular tissue with the exception of bulbar conjunctiva, which is commonly examined in contact lens studies. Curved scissors are the preferred instrument. A 0.5–1 cm portion of the optic nerve, should be retained with the globe.

The enucleated globe and extraocular tissues need to be submerged in fixative at a ratio of at least 1 part globe to 10 parts fixative. Wide mouth containers should be used and gauze may be placed on top of the globe to ensure complete submersion. The globes and extraocular tissues need to be identified as being from the right eye (OD) or from the left eye (OS) and tattoo ink or tissue dyes may be used to help in orientation for trimming.

There are many different fixatives that may be used for ocular fixation but none of them is ideal. Each histotechnology laboratory tends to have a preference for ocular fixatives. When using a particular laboratory, use the fixative that they prefer to help minimize artifacts that can occur when experimenting with a different fixative. Commonly used fixatives include glutaraldehyde, a mixture of glutaraldehyde and formalin, Davidson's fixative, modified Davidson's fixative, Bouin's fixative and Zenker's fixative, although others are used.⁵⁸ Adequate fixation generally requires approximately 48 h of initial fixation, possibly followed by an additional 24 h fixation in 10% neutral buffered formalin to ensure that the globe is firm enough for trimming and that the lens is fixed completely. Fixatives such as glutaraldehyde alone or mixed with formalin need to be exposed to the internal aspect of the globe for adequate fixation of the vitreous body and retina. Intraocular fixation is enhanced at the time of the initial fixation, by either slowly injecting the fixative into the vitreous cavity, or waiting for 5–30 min of initial fixation and then creating a small window. The injection site or the window should not be the plane of section for microscopic examination. The other fixatives mentioned do not need the use of injections or windows because they adequately penetrate all portions of the globe.

After adequate fixation, globes for paraffin-embedding are trimmed. The trimmer should be aware of any clinical ophthalmic findings to ensure that trimming will result in tissue sections along a plane for microscopic correlation. Trimming requires the use of a long, sharp blade or disposable microtome blade in order to get a smooth cut with minimal distortion. The use of a razor blade requires a sawing motion that may potentially cause artifactual retinal separation. When trimming globes, the initial cut should be along the desired plane of section and the second cut should create a hole that is deep enough to ensure adequate embedding, but ideally shallow enough to avoid hitting the lens. This is accomplished by using megacassettes, and the tissue is ready to be processed.

Processing involves the infiltration of tissues with paraffin. Following processing, the paraffin-infiltrated tissue is embedded in paraffin blocks. Sections of tissue cut at a thickness of 3–6 microns are then applied to glass slides and stained in preparation for microscopic examination. The routine stain used is a combination of hematoxylin and eosin, but additional stains may be used to highlight certain ocular structures or tissue reactions. For example, periodic-acid-Schiff stain is used to highlight basement membranes

(e.g. Descemet's membrane) and a Masson's trichrome is helpful in evaluating fibrosis.

TEM and SEM are occasionally used in the examination of retrieved implant or tissues previously implanted with medical devices, but the decision to use these techniques needs to be made when the protocol is prepared. Since preservation of the tissue is essential, systemic perfusion with an appropriate fixative may be needed at the time of enucleation and the tissue samples collected for TEM should be very small to minimize artifacts.

Tissues not specifically prepared for TEM are usually not useful. If the study involves examination of intraocular lenses, then a portion of the lenses may need to be examined by use of SEM.⁵⁹

Tissue responses of the eye are generally dependent upon the location of the medical device. The following are a few examples of pathologic findings. Contact lenses may cause topical irritation to the corneal epithelium, which may result in vascularization of the corneal stroma, inflammatory cell infiltration in the episclera of the corneoscleral junction (*limbus*), and an inflammatory cell infiltrate in the bulbar conjunctiva. Medical devices that extend into the anterior chamber might cause physical destruction of corneal endothelial cells and the proliferation of fibrous tissue (retro-corneal membrane), obstruction of the filtration angle, and adherence of the iris to the cornea (anterior synechia). External contact with the lens might result in opacification and cataract, but disruption of the lens epithelium within the anterior lens capsule results in proliferation and fibrous metaplasia of the cells to form fibrous plaques. Implants in the choroid may cause hemorrhage or a disturbance of blood flow, thus decreasing nutrition to the outer retina (photoreceptors) and causing retinal degeneration, detachment, or both.

Microscopic examination is only a portion of the assessment of ocular medical devices, and microscopic findings need to be correlated to results from the *in vivo* assessment of ocular irritation and toxicity.⁵⁷

19.8.6 Clinical correlates

Generally, *in vivo* assessment involves a routine ophthalmic examination performed by trained and qualified personnel with or without the use of specialized equipment. The basic clinical ophthalmic examination involves direct ophthalmoscopy and indirect ophthalmoscopy, which includes an examination of the ocular fundus through a dilated pupil (funduscopy). When a medical device involves the structures of the anterior segment (i.e. bulbar conjunctiva, cornea, anterior chamber, iris and lens), in-life examination usually involves slit-lamp biomicroscopy. The slit-lamp illuminates and magnifies ocular structures to allow for the detection of minute morphologic changes. Stains may be used with the slit-lamp. Rose Bengal stain helps

in identifying degenerating epithelial cells and fluorescein stain is used to evaluate the precorneal tear film or assist in the detection of corneal ulcers. There should always be an attempt to correlate clinical ocular findings with microscopic ocular findings.

There are several non-routine, non-invasive, ophthalmic tests that may be used to help characterize ocular changes associated with ocular implants.⁵⁷ Assessment of the cornea may involve esthesiometry, which tests the sensitivity of the cornea, pachymetry, which measures the thickness of the cornea, keratometry, which measures corneal curvature, and specular microscopy, which is used to visualize the corneal endothelium. Intraocular pressure can be evaluated with various types of tonometers and the filtration angle can be evaluated through gonioscopy. Lenticular changes can be characterized through Scheimpflug imaging and multiple structures of the eye, especially in the posterior segment, may be imaged by use of optical coherence tomometry. Electroretinography (ERG) is a way to measure the functional status of the retina. There are different types of ERG and changes in the recordings of the ERG may or may not have morphologic microscopic correlates.

Ocular medical devices include those that are topical, such as contact lenses, and those that are implantable, such as intraocular lenses. To fully understand the potential for irritation, local toxicity and occasionally systemic toxicity, requires a thorough clinical evaluation with or without special techniques and a thorough microscopic examination with or without special stains, specific embedding media, or electron microscopy. Microscopic findings should be correlated to clinical observations for a complete understanding of ocular effects. Information on device biocompatibility testing can be found in the documents provided by the International Organization for Standardization (ISO).⁶⁰⁻⁶²

19.9 Conclusion

The understanding of clinical findings is better with microscopic correlations, and an understanding of many microscopic findings is aided by electron microscopic analysis. Determination of these correlations helps to ensure adequate monitoring, correct interpretation, and better understanding of the findings. With the variable biomaterials used for implantable medical devices, it is important to choose the best approach to evaluate each one. On the other side, the use of *in-life* imaging techniques will allow reconstruction of a full device–host interaction and complement the microscopic description of the different events.

Emphasis was placed on selection of biological and material controls, as well as selection of appropriate methods for specimen preparation and evaluation to prevent misinterpretation. Advances in morphologic analysis⁶³ continue to enhance our understanding of biological responses and material changes.

A wide range of interactions occurs between living tissues and biomaterials. Beneficial or adverse effects may be observed. Microscopic examination of implanted sites is one of the most important methods for the assessment of cellular and extracellular events that comprise the host response, and is important in determining if and what additional testing is necessary.

Minimal local biological response does not negate the potential for systemic effects (endovascular device with thromboembolism, toxicity associated with leachable components). A harmonious implant-tissue interaction at time T can convert into an undesirable reaction at $T + n$ due to material change (alteration, degradation) in the given biological environment. A deleterious local reaction to an implant does not necessarily mean that the implant is not qualified. Immune reactions linked to the animal species used (e.g. implant combined with a human derived proteins tested in pig) can result in xenogenic reactions and may require the use of immunodeficient animal models to better define tissue reactions.

Understanding of pathological and healing processes and knowledge of the physical properties and composition of materials are critical to assessing biocompatibility of medical devices. Biocompatibility, as defined by the consensus conference of the European Society of Biomaterials held in 1986,⁶⁴ includes the notion of performance of the medical device. The ability of a material to perform with an appropriate host response in a specific situation defines the term of biocompatibility. Hence, microscopic evaluation of safety and performance are interdependent and complementary. That is why the description and the grading of safety findings, as defined for example by the ISO 10993-6, should develop in a direction taking into account performance criteria, yet still remaining part of the safety evaluation. For example, a bio-textile designed for tissue reinforcement can elicit a minimal tissue reaction without significant signs of tissue ingrowth, which corresponds to suboptimal implant performance and unsafe medical device.

Microscopic quantification of safety and performance findings is one of the most challenging aspects for the future, particularly with regard to soft tissues. The use of new microscopic techniques,⁶⁵ such as digital pathology and quantitative imaging, will contribute to integrating information on safety and performance of implants. This new perspective should help achieve a more accurate microscopic assessment and understanding of the host response to implanted medical devices.

19.10 Acknowledgments

The authors wish to express their gratitude to Dr Rosy Eloy for her extensive and valuable suggestions and inputs, and to Dr Richard Wallin for reviewing the entire chapter and offering useful advices. We want to warmly thank the following companies for their pictures that help illustrate this

chapter (Galderma, Cartiheal Ltd Israël, Stryker, nvt GmbH and Johnson & Johnson MEDICAL GmbH). We would also like to thank the highly skilled staff of our lab (Joe Saby and team, Carine Wirth, Vincent Boiza and team).

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